PROCEEDINGS OF THE FOURTH
INTERNATIONAL CONGRESS OF THE

INTERNATIONAL SOCIETY OF HEMATOLOGY

MAR DEL PLATA, ARGENTINA—SEPTEMBER 20-27, 1952

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Preface

THE INTERNATIONAL SOCIETY OF HEMATOLOGY, which alternates its biennial meetings between the hemispheres, met in September 1952 in the Western Hemisphere—more specifically, in Argentina. There at Mar del Plata, an "Atlantic City" type of resort, gathered more than 300 hematologists, most of them from Latin America but with a generous sprinkling of Europeans, North Americans, and some others including a representative of the Japanese Society of Hematology (500 members). Principal addresses, short communications and round table discussions were carried out amidst the plush though massive surroundings of the Hotel Provincial next to the great Casino, said to be the world's largest. With the care given by the local Committee so ably headed by the President of the Congress, Alfredo Pavlovsky, the Congress ran like clockwork and was undoubtedly a great impetus to the further development of hematology in South America.

This volume contains most of the formal material presented at Mar del Plata although the informal symposia and the important knowledge gained through friendly face to face discussions are inevitably lacking. The principal addresses are for the most part presented in full. Of necessity, the numerous communications are presented in abstract form and will undoubtedly be published elsewhere. It is likely that future proceeding volumes will consist in their entirety of abstracts, an advantage not only from the standpoint of possible publication in various journals but from that of financial outlay. Nevertheless much of the material in the present volume is original and in large part written in Spanish, which should have considerable appeal for our Latin and Latin American group.

Mr. Henry M. Stratton, President of the publishing house of Grune & Stratton, is to be commended for his continued interest in hematology in general and in our Society in particular. Without his willingness to forego financial gain, publication of this volume would have been impossible. Credit should also be given to two young bi-lingual Latin American physicians, Dr. Jorge Lajous and Dr. Gonzalo E. Aponte, who spent many hours on translations and proofreading. As a small token of appreciation, they have been placed on the list of Assistant Editors.

WILLIAM DAMESHEK

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PART I

Neuroendocrinologic Regulation of Hematopoiesis and Hemostasis

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Regulacion Neuroendocrina de la Hematopoyesis y la Hemostasia

COMMUNICATIONS

El Factor Vascular en las Enfermedades Hemorragíparas

MARIANO R. CASTEX*

EL ESTUDIO del factor vascular en la etiofisiopatogenia de las enfermedades hemorragíparas ha sufrido a través del tiempo las mismas vicisitudes— si bien con plazo mucho más limitado—que el del factor vascular en la hemostasia, ambos procesos, hemorragia en las enfermedades hemorragíparas y hemostasia, estrechamente conexionados. La hemorragia por heridas voluntarias, intencionales, accidentales, traumaticas, etc., es de observación prácticamente continua o casi de incidencia casi ininterrumpida; no así el accidente hemorragíparo de las enfermedades hemorrágicas de incidencia considerablemente menor y a aparicion generalmente espontanea, pero no excepcionalmente inducido accidentalmente por influjos físicos, señaladamente traumas mecanicos violentos o leves, contundentes o vulnerantes y ocasionalmente por de cargas emocionales, sensitivoafectivas desplacenteras por lo comun y a forma unica intensa o iterada o sostenida.

En su excelente obra "La fisiologia y patología de la hemostasia" de 1951 Quick estudia el factor "contraccion vascular".

Recuerda que Petit, en 1731, sostuvo que la hemorragia se detiene merced a la formación del coagulo sanguineo y que Morand, cinco anos mas tarde, en 1736, sostuvo que las arterias se contraen en sentido longitudinal formando pliegues y reduciendo la luz vascular. Algunos anos más tarde John Hunter expresó que todo vaso injuriado tiene tendencia natural a contraerse y gran numero de distinguidos cirujanos ingleses aceptaron en ese siglo la teoria de la contraccion vascular en la hemostasia.

Ya en 1805 Jones alia ambos factores en su concepto de la hemostasia, considerando a la formacion del coagulo y a la contracción y retracción vascular como fenómenos sucesivos y convergentes.

Los adelantamientos realizados en el estudio de la coagulación de la sangre en la segunda mitad del siglo XIX hicieron poco menos que abandonar la teoria vascular. Pero observaciones en el ser humano y hechos experimentales llevados a efecto volvieron a resucitar el factor vascular, que se enriquecio con conceptos que fueron muy discutidos acerca de la "adhesividad de los endotelios vasculares" y de la "acción vasoconstrictora de las plaquetas desintegradas".

En la notable obra "Las enfermedades hemorrágicas" de Baserga y de Nicola de 1950 Povatti colabora en el capítulo de la hemostasia. Estudian sucesivamente la serie de factores que intervienen en el complejo proceso: 1) la *formacion del trombo*, 2) *los fenomenos de adhesividad de los endotelios* puestos de relieve entre otros por Roskam (1921) y Herzog (1925), 3) *los fenómenos mecánicos pasivos* señalados para las arterias por Marchand (1900).

* Miembro de la Academia Nacional de Medicina de Buenos Aires, Argentina.



MICROFOTOGRAFÍA 1—OBSERVACIÓN 1

FIGURA 1 MICROFOTOGRAFÍA 1 —Cuerno lateral de médula espinal normal

PRIMER CASO Hombre de 56 años de edad *Peliosis reumática purpura bilateral simétrica* a nivel del tractus intermedio lateral de ambos lados y en diferentes cortex seriados examinados para excluir la alternancia fisiológica se observa

1) Evidente rarefacción celular reducción considerable del numero de las mismas (microfotografia 2)

2) Pronunciada disminución de volumen de las escasas células existentes (microfotografía 2)

3) Excentricidad nuclear en la mayoría de las células (microfotografías 2 3 y 4)

4) Retracciones del cuerpo celular (microfotografías 2 3 y 4)

5) Cromatólisis central con acumulación de las granulaciones cromáticas en el limbo celular (microfotografías 3 y 4)

6) Disminución del número de prolongaciones celulares

En suma todas las alteraciones que caracterizan al estado llamado de esclerosis celular

Microfotografia 5 Coloración Spielmeyer pequeno aumento enrarecimiento de fibras en los cordones antelaterales en la vecindad del cuerno posterior y de la raíz correspondiente

Microfotografía 6 Coloración hematoxilinaeosina exagerada vascularización de los cuernos posteriores

Microfotografía 7 Comisura gris en vecindad del asta lateral 5 vasos de paredes espesadas con comienzo de degeneración hialina

Microfotografía 8 Método de Nissl célula normal del asta anterior pero aun en esa región espinal existen células marcadamente alteradas

En síntesis Presencia de sendas lesiones celulares en los centros simpáticos espinales que con grandes probabilidades han sido decisivas en la patogenia del purpura reumatoideo o sindrome de Schoenlein

(1952) por parte de Blackmann Cohen y Watson y de Meachen Orbison Heinle Steele y Schraefer los cuales la consideran afección primaria degenerativa intramural de arteriolas y capilares vinculada con las colagenosis

Corresponde de momento recordar la acción directa y selectiva del oxido de carbono sobre el sistema nervioso central y sobre determinadas areas del mismo en particular como ser las estructuras de los nucleos grises de la base que integran

Krogh (1917) Haberer (1918) Schmidt (1930) Lexer (1934) y designados *Función de prevención* por Apitz (1945) y a nivel de los capilares por Stephan (1921) y Heimberger (1926) 4) *los fenómenos de contracción activa* observados directamente en el hombre por Kirkland (1763) Magnus (1932) Bier (1933) y bajo forma de espasmos por contusiones por numerosos investigadores entre otros Krogh (1917) Kuttner y Baruch (1920) Leriche (1921) Stubel (1922) Tanennberg y Herrmann (1927) Apitz (1942) Chen y Tsai (1944) Zucker (1947) En la génesis de este factor de vasoconstricción debe concederse importancia a las sustancias vasoconstrictoras liberadas en la desintegración de las plaquetas descubiertas por Stewart y Zucker (1943) y confirmadas y ampliadas por las investigaciones de Le Sourd y Pagniéz (1914) Janeway Richardson y Park (1918) Roskam (1927) Quick (1942) Reid (1943) Zucker (1947) 5) *las reacciones vasculares en otros distritos alejados del sitio de la hemorragia* ampliamente estudiados por Apitz en 1942 y por Zweifach Chambers Lee y Hyman (1948) y por Shorr Furchgott y Zweifach (1948) los que establecen mediante la hemorragia experimental una fase de vasoconstricción periférica debida a *sustancia vasopresora nefrógena seguida de una fase de vasodilatación provocada por una* sustancia vasodepresora originada al parecer en el hígado y en los músculos la que según las investigaciones de Mazur y Shorr (1948) sería un complejo de ferritina apoferritina 6) y último consideran *a la integridad anatómica del vaso* como condición fundamental para la prevención de la hemorragia Recuerdan que Klemperer en interesante ponencia de 1948 se formula la pregunta ¿dónde y cómo escapa la sangre de los vasos? y que Humble en 1949 establece mediante sus investigaciones capilaroscópicas que la hemorragia ocurre en la porción distal de la arteriola eferente al asa capilar

Hemos sintetizado los factores que intervienen en la hemostasia por ser los mismos que participan en la génesis de las manifestaciones hemorragíparas en las enfermedades o diátesis hemorrágicas

Baserga y de Nicola en su importante tratado de 1950 clasifican las enfermedades hemorrágicas en aquéllas de origen discrásico sanguíneo y en las que no proceden de este origen siguiendo en ello la orientación corriente en la medicina interna de lo que va del siglo bien destacada en la importante publicación de R Jurgens de 1949 sobre diátesis hemorrágicas

En su monografía Patogenia y terapia de las diatesis hemorrágicas ' de 1923 expresa W Schultz que la lesión vascular domina la patogenia de las hemorragias en las avitaminosis y en los tipos clínicos infectivos polifocales

Recuerda que según Glanzmann las hemorragias en las purpuras infectivas se deben a lesiones vasculares anafilactoides por acción tóxica sobre los vasos cual ocurre en la urronozis o enfermedad de suero La acción tóxica directa sobre los vasos en los estados hemorragíparos es lícitamente admisible pues ella ocurre en la intoxicación por el óxido de carbono

En la purpura trombopénica a la trombopenia se asocia una lesión vascular causa de las manifestaciones hemorrágicas

En la purpura atrombopénica esencial agrega W Schultz en 1923 la distribución simétrica de las manifestaciones purpúricas cutáneas sugiere la intercalación patogénica del aparato nervioso

La purpura trombopénica trombótica descrita clínicamente por Moschowitz en 1925 estudiada histológicamente por Baehr Klemperer y Schifrin en 1936 y ulteriormente por numerosos autores es un *síndrome de diátesis hemorrágica febril* con anemia grave trombopenia *con discrasia vasculosanguínea intumescencia hepatoesplénica y manifestaciones neurales* objeto de recientes estudios



MICROFOTOGRAFÍA 5—OBSERVACIÓN 1

portante que sea esta intervención parece requerir la sinergia íntima con el conjunto de factores reguladores de la coagulación de la sangre

Dejando de lado todo lo concerniente al factor sanguíneo nos concretaremos al estudio del factor vascular que al parecer domina la patogenia del fenómeno hemorrágico en algunas enfermedades hemorrágicas factor vascular cuya anormalidad puede obedecer a dos mecanismos diferentes la lesión de la estructura vascular o al trastorno funcional del vaso de índole neural

El conocimiento *del influjo trofodinámico del sistema nervioso sobre los vasos sanguíneos* producto de observaciones clínico anatómicas y experimentales remonta a casi un siglo atrás

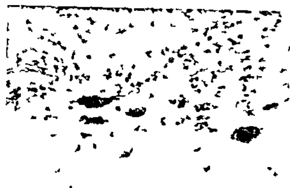
Rokitansky en 1859 describió la gastromalacia gelatinosa y *hemorrágica* en niños y adultos fallecidos por afecciones orgánicas del encéfalo

Schiff y Ebstein en 1867 Brown Sequard en 1876 von Recklinghausen en 1883 refieren una serie de *trabajos experimentales* en los que mediante lesiones de determinadas areas del sistema nervioso medula espinal tronco cerebral puente ganglios cervicales etcétera ocurrieron *hemorragias espontaneas* en organos diversos estomago pulmón suprarrenales etc

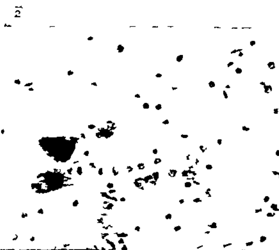
Testut en 1876 actuando sobre el nervio ciático y la médula espinal consiguio equimosis en las estructuras externas del cuerpo del animal utilizado

Weir Mitchell en 1869 considera a la purpura como una neurosis por la distribución simétrica de las equimosis y por los dolores debida a la debilitación de las paredes vasculares con permeabilización de sus estructuras por mecanismo neural Despunta así Weir Mitchell como precursor de la fisiopatogenia nerviosa de la purpura que atribuye *al aumento de la fragilidad y permeabilidad vascular por mecanismo nervioso*

Henoch en 1874 destaca el influjo del sistema nervioso en la génesis de la purpura reumatoidea Couty en 1876 atribuye la purpura a lesiones del simpatico y del plexo solar Faisans en 1882 dedica su tesis doctoral de Paris a *la purpura mielopática* que atribuye a la hiperemia de los haces posteriores de la



MICROFOTOGRAFÍA 2—OBSERVACIÓN 1



MICROFOTOGRAFÍA 3—OBSERVACIÓN 1



MICROFOTOGRAFÍA 4—OBSERVACIÓN 1

el sistema e triado asunto éste ampliamente e tudiado en la tesi doctoral de nuestro di cipulo y colaborador Dr Carlo Guerra en el ano 1930

C Bickel dedica en 194ɔ un importante trabajo al Papel de las alteraciones va culares en la patogenia de las diate is hemorragicas manifiesta en el que no existe enfermedad hemorragípara en la que no intervenga el factor va cular ya sea a título dominante ya sea en forma acce oria agregando que por un



MICROFOTOGRAFÍA 5—OBSERVACIÓN 1

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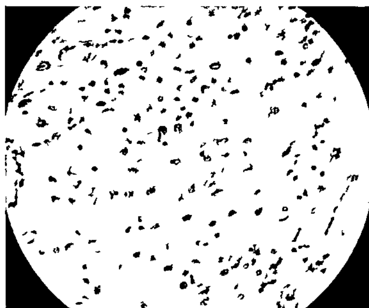
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MICROFOTOGRAFÍA 6—OBSERVACIÓN 1

médula considerando posible que el reumatismo origine la purpura por vía indirecta neural exclusiva

En las tesis doctorales de Paris de Mathieu en 1883 y Du Castel en 1886 figura en la clasificacion de las purpuras la forma clinica de origen nervioso

Singer y Pattani publican en 1884 un caso de piloroespasmo y hematemesis con el vago englobado dentro de ganglios bronquiales tuberculosos

Lapinsky en 1899 por la seccion del ciatico en el perro observo variaciones en el calibre de los vasos en la velocidad sanguinea y en la presión y comprobo la supeditación de ciertas alteraciones vasculares a la neuritis dilatacion de arterias y venas flexuosidad de las mismas y por fin la aparicion de hemorragias

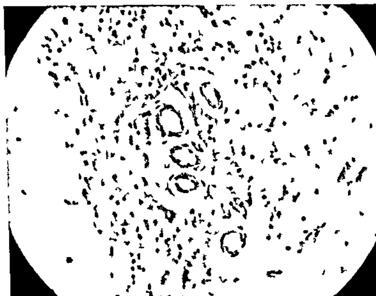
Vilmain en su tesis de Paris de 1902 atribuye la purpura a incrementada irritabilidad de los centros vasomotores por toxinas la cual originaria *intensa vasodilatacion capilar seguida de desgarramiento vascular*

Grenet en su tesis de Paris de 1904 consigna observaciones clinicas de purpuras simétricas en afecciones meningeas y de herpes zoster y por ello considera frecuente la participacion del sistema nervioso en la patogenia de todas las variedades de purpuras

Goldstein y Nicolesco publican en 1904 un caso de hematemesis en el decurso de una hemorragia talamica

Delearde y Halley en 1912 admiten la intervencion del sistema nervioso en la patogenia de la purpura cronica simple pero con la mayoria de los autores conceden maxima importancia a *la lesion de la pared vascular* en el sentido de un *incremento de la fragilidad de los pequenos vasos perifericos* cuyo *desgarramiento* se favoreceria *por trastorno vasomotores*

En 1927 aparece la monografia de Schindler *Sistema nervioso y hemorragias*



MICROFOTOGRAFÍA 7—OBSERVACIÓN 1

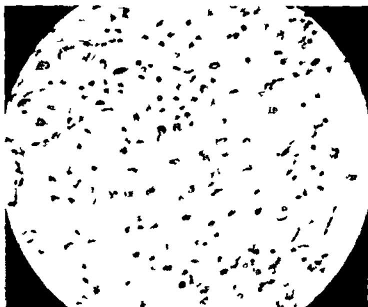
espontáneas exhaustiva del asunto con ejemplario de observaciones clínicas agenas y propias sobre *equimosis espontaneas en las afecciones organicas mas variadas del sistema nervioso central y periférico* y al propio tiempo *con afecciones funcionales* complementando su excelente y completísimo trabajo con toda la informacion recopilada respecto a las equimosis espontaneas en la neurastenia (Houeix de la Brousse 1898) en la histeria (Gilles de la Tourette 1890) así como también lo concerniente a la inducción de equimosis por hipnosis (Ferrand 1890 Pemond 1891) Insiste Schindler en 1938 sobre el mismo asunto refirmando e en las mismas conclusiones *las manifestaciones de purpura pueden aparecer en la lesion de cualquier region del sistema vasomotor Las hemorragias en las diatesis hemorragíparas son síntoma de vasomotores lesionados y ese daño puede afectar cualquier porcion del sistema vasomotor En todo caso de purpura corresponde precisar el agente injuriante y el sitio del sistema nervioso por él afectado*

En 1934 Masten y Bunts publican ocho casos de gastromalacia ufusiones hemorragicas y perforaciones gastricas en sujetos afectados por variados procesos organicos del encéfalo

Keller en 1936 informa del resultado de sus experiencias sobre hemorragias y ulceracion gastrica inducidas por lesiones neurovegetativas

W R Hess (1949) en sus investigaciones sobre lesiones experimentales del diencéfalo comprobo en un animal la aparición de multiples erosiones hemorragicas en el estómago

Wanke en 1939 estudia la hemorragia pulmonar neurovegetativa y en el mismo ano 1939 ponen de relieve Davis y Wilson la *gravitacion del influjo psíquico en la génesis de las hematemesis y de la perforacion de la ulcera gastroduodenal* hecho que sería plenamente confirmado en 1940 con la experiencia clínica recogida en Inglaterra durante la Blitzkrieg teutona



MICROFOTOGRAFÍA C.—OBSERVACIÓN 1

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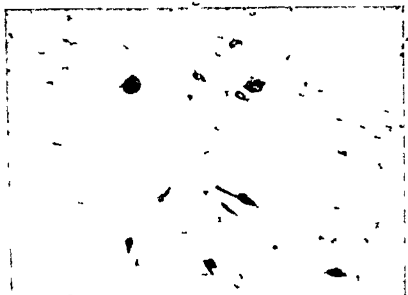
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En 1927 aparece la monografía de Schindler: *Sistema nervioso y hemorragia*



MICROFOTOGRAFÍA 9—OBSERVACIÓN 2

SEGUNDO CASO *Purpura generalizada en tronco y extremidades a distribución simétrica*

1) Intensa rarificación celular con considerable reduccion en el número de células que constituyen el tracto intermedio lateral o nucleo simpático láterosuperior de ambos lados para excluir la alternancia fisiológica (microfotografía 9)

2) Acentuada disminución del volumen de las escasas células existentes a nivel de los núcleos simpáticos láterosuperiores (microfotografía 9)

3) Excentricidad nuclear en la mayoría de las células que integran el nucleo intermedio látero superior y disminucion del numero de prolongaciones celulares (microfotografías 10 11 y 12)

4) Alteraciones vasculares gruesas evidentes en el espesamiento de la pared vascular con degeneracion hialina incipiente coloración hematoxilina eosina (microfotografía 13)

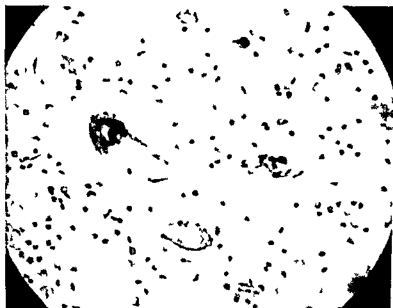
En suma Profundas alteraciones celulares en los integrantes de los nucleos simpáticos láterosuperiores

nervioso y las paredes vasculares logro la estancacion circulatoria y la salida de entrocitos desde los capilares hecho confirmado por Ricker quien lo explotó eficazmente para la produccion experimental del infarto hemorragico extenso del riñon concluyendo que *la estancacion con hemorragia capilar responde a la perdida de la excitabilidad nerviosa que origina la inhibicion funcional de los capilares*

Reilly y colaboradores del Hospital Claudio Bernard de Paris bien conocidos por sus notables trabajos experimentales en el dominio del sistema neurovegetativo (citados por Langeron y Wolf en 1949) crearon experimentalmente en el animal una afección semejante a la purpura reumatica con idénticas lesiones cutaneas abdominales y renales por procedimientos diversos todos los cuales *lesionaban territorios variados del sistema nervioso simpático y engendraban dilatacion capilar seguida de desgarramiento y extravasacion sanguínea*

De todo lo expuesto corresponde poner en destacado relieve los siguientes hechos fundamentales

1) *Que la clinica humana* ha demostrado la aparición de manifestaciones he



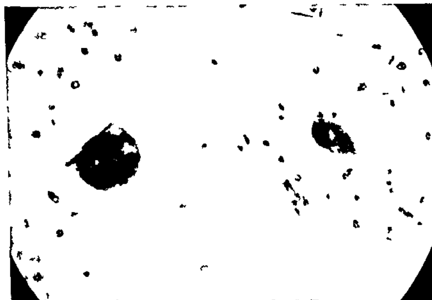
MICROFOTOGRAFÍA 8—OBSERVACIÓN 1

La investigación anatomopatológica del sistema nervioso central en la púrpura del ser humano se limita a tres publicaciones: de Gordon en 1919, quien comprueba intensa vacuolización a nivel del sistema nervioso central, estrictamente limitada a la sustancia gris de la médula espinal; de nosotros con nuestros discípulos Nicolás Romano, Armando Camuer y Ramón Lorenzo en 1923, comprobando en dos casos de púrpura crónica simétrica a nivel de los cuernos laterales de la médula dorsal—asiento de los centros vasomotores que integran el haz intermedio lateral simpático dorsolumbar—disminución del número de las células, reducción del tamaño de las escasas células existentes, retracción del cuerpo celular, cromatolisis central con acumulación de granulaciones en la periferia, disminución del número de dendritas; en suma, todas las alteraciones que caracterizan la esclerosis celular; y de A. Frochlich en 1938, en un caso similar a los nuestros, comprueba lesiones en los cuernos laterales espinales idénticas a las comprobadas por nosotros en 1923, lo cual lleva a Frochlich a decir que su observación confirma las nuestras y que la lesión del sistema nervioso puede determinar la localización de la púrpura y que ella parece indispensable para la génesis de la púrpura, pues en todo momento se lograba hacer aparecer la manifestación cutánea mediante el leve traumatismo.

En lo que concierne a la *producción experimental de la púrpura*, haciendo abstracción de las equimosis en las estructuras externas logradas por Testut en 1876 y por Lapinsky en 1899, mediante la acción experimental sobre la médula y el ciático en el perro, ella se limita a las investigaciones de Grenet, de Natus y Ricker y de Reilly.

Grenet (1903–1904) provocó púrpura en animales con hígado previamente lesionado mediante la inyección de tóxicos dentro de la médula espinal.

Natus en 1910, en animales (conejos), *mediante la interrupción entre el sistema*



MICROFOTOGRAFÍA 11—OBSERVACIÓN 2

Para la completa interpretación etiofisiopatogénica de todos estos hechos corresponde 1) Recordar brevemente la anatomía del sistema nervio o vaso motor 2) Considerar el estado de los vasos en las diversas diatesis hemorrá gicas 3) Contemplar la fisiología normal y patológica del sistema arteriolo capilar a la luz de las trascendentes investigaciones de los últimos años

1) *El sistema nervioso vasomotor* (L. I. Mueller) está integrado por tres centros con tono autónomo

a) *Centro craneal* en las paredes del tercer ventrículo del hipotálamo y del tálamo b) *Centros espinales* en la zona intermediolateral desde el octavo segmento cervical hasta el tercer segmento lumbar (núcleo simpático laterosuperior) y en la región lumbar inferior y sacra (núcleo simpático lateromedial inferior) c) *Centros periféricos* en las paredes vasculares constituyendo ricas redes anastomóticas

La corteza de los lóbulos frontales con rica repre entación autónoma ejerce influjo inhibitorio sobre los centros vegetativos hipotalámicos por intermedio del haz neocórtico septalis demostrado por Watts y Fulton

Las fibras vasodilatadoras pertenecen al parasimpático arrancan aparentemente del cuerno posterior y transitan a través de los ganglios espinales y no de las estructuras simpáticas *Las fibras vasoconstrictoras* pertenecen al simpático C H Cicardo (1951) mediante su exploración por excitantes químicos coincide en la localización de sus centros en los cuernos laterales dorsolumbares Las fibras vasoconstrictoras abandonan la médula por las raíces anteriores unidas con el hacecillo que arranca del ganglio espinal abandonan el nervio espinal por la rama comunicante blanca y se dirige a la cadena ganglionar simpática Desde las células multipolares ganglionares parten los nervios postganglionares por las ramas comunicantes grises y llegan a la vía sensitiva del nervio espinal Este recorrido se refiere únicamente a las extremidades y a la piel del tronco Para los vasos sanguíneos de las dos grandes cavidades las fibras van directamente y no por vía de lo nervios espinales

2) *El estado de los vasos sanguíneos en las diversas diatesis hemorrágicas ha sido objeto de innumerables investigaciones*



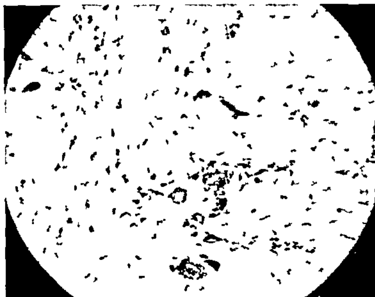
MICROFOTOGRAFÍA 10.—OBSERVACIÓN 2

morrágicas viscerales y cutáneas en las más variadas afecciones orgánicas del sistema nervioso central y periférico: a distribución simétrica en extremidades y tronco y hasta con distribución radicular o periférica o con localización exclusiva en segmentos corporales afectados o paralizados; aparición de equimosis seguidamente a dolores fulgurantes de la tabes (Strauss, Schaffer); viruela exclusivamente *hemorrágica en los miembros paralizados en un caso de esclerosis en placas* (Chevalier, 1877).

2.) *Que la investigación histológica* (M. R. Castex, N. Romano, A. Camauer, R. Lorenzo, 1923; Froehlich, 1938) ha revelado sendas lesiones microscópicas exclusivamente localizadas en las constelaciones celulares que integran los centros vasomotores del núcleo intermediolateral en los cuernos laterales espinales.

3.) *Que la inquisición experimental* (Natus y Ricker, Reilly y colaboradores) ha demostrado que en la interrupción entre el *sistema nervioso y las* paredes vasculares ocurre la estancación sanguínea y la salida de eritrocitos de los capilares, y asimismo que la irritación variada en diversos territorios del sistema nervioso simpático origina dilatación capilar y ruptura de los mismos secundaria a vasoconstricción arteriolar, *o en otros términos que las alteraciones del sistema simpático o vasomotor pueden originar hemorragias cutáneas o viscerales a través de una disfunción o discrasia vascular*.

Conviene señalar que en las dos observaciones propias la púrpura fué crónica y las lesiones en los *núcleos del haz intermediolateral dorsolumbar* fueron intensas y de tipo irreversible, mientras que en la observación de Froehlich, con lesiones espinales similares, se lograba hacer aparecer la púrpura a voluntad mediante leve traumatización de la piel, hecho que evidencia un estado capilar frágil a nivel de las zonas así provocadas.



MICROFOTOGRAFÍA 13—OBSERVACIÓN 2

resultados de Ledingham y Bedson acerca de la imposibilidad de producir la purpura mediante la trombocitopenia pura y establecieron que el suero antiplaquetas engendra la purpura por medio de un efecto vascular capital y no por accion sobre las plaquetas

Las investigaciones de Pick Rosenthal y Erf (1937) de Meyer y Chaffee (1941) de Chambers y Zweifach (1944) establecen que se requiere la alteración de la pared vascular para la rotura del vaso La incrementada fragilidad capilar puede deberse a una anormalidad o a una defectuosa elaboracion del cemento a alteraciones de las estructuras pericapilares de sostén señaladamente de los elementos elásticos quizas por accion de la enzima hialuronidasa sobre esas estructuras ricas en acido hialuronico que determinaría la licuacion del tejido de sostén distendiéndose primero y rompiéndose luego el vaso por accion de la presion intracapilar

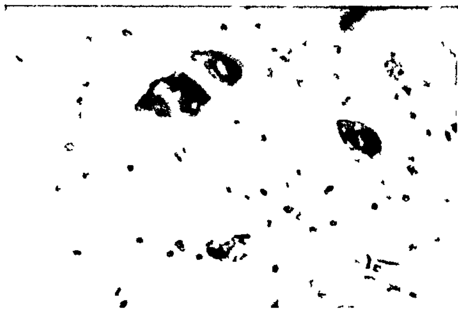
Asboe Hansen sostiene en 1950 que las mastzellen segregando acido hialurónico transmiten actividad humoral al tejido conectivo

Whitesell y Snell ponen de relieve la trascendencia de la fragilidad capilar incrementada asociada a trombopenia señalada por Snell Vanzant y Judd en 1930 y por Morlock y Hall en 1943 y a la hipotrombinemia en la génesis de las hemorragias en las afecciones hepáticas

El *problema de la fragilidad capilar incrementada o de la resistencia disminuida* ha sido objeto de numerosísimas investigaciones

La multiplicidad de las técnicas de investigacion de la fragilidad capilar es prueba de su insuficiencia ninguna puede considerarse rigurosamente científica escribe Bickel en 1945

El profesor Antenor Rey de Cordoba dedica en 1940 una excelente y completa monografía a la Fragilidad capilar normal humana destacando su importancia en la clínica y en 1949 los Drs Alfredo V Di Cio y L Klein del Instituto de Investigaciones de la



MICROFOTOGRAFÍA 1.—OBSERVACIÓN 2

Ano atra sentido Brocq *la fragilidad de los vasos* en la purpura el simple rascamiento puede bastar para inducir la aparicion de la purpura

Nosotros en ilamos la hemorragia capilar en mapa y a distancia en un caso de periarteritis alérgica de hombro con hipotrombinemia por aspirina en el que la suave frotacion origino una considerable hemorragia difusa en toda la piel del hombro y brazo afectado y coetaneamente una pequena hemorragia en el cuerpo vitreo homolateral (1950)

Vilman en 1902 y Dekarde y Halley en 1912 sostuvieron que no bastaba la alteracion del sistema nervioso sino que con ella se requería la alteracion de la pared vascular para engendrar la manifestacion hemorragica

Kroemcke en 1926 exige en la patogenia de la purpura la trombopenia el alargamiento del tiempo de sangría y la lesion de la pared capilar

El mismo ano 1926 afirma Bedson que el descenso o ausencia de plaquetas no basta per se para engendrar una purpura

En 1927 expresa Schindler que solo hay acuerdo en que las diversas hemorragias espontaneas responden a lesiones de la pared vascular ignorando el la índole de las mismas recordando al propio tiempo los fundamentales investigaciones experimentales de la escuela de Ricker que establecieron que *la hemorragia espontanea surge cuando cesa la conexion funcional entre el sistema nervioso y la pared vascular*

En el analisis anatomopatologico de 355 casos de diátesis hemorragicas Feldman y Fox refieren en 1941 haber comprobado en el 10 por ciento anormalidad de la coagulacion en el 22 por ciento déficit de plaquetas y en el 68 por ciento alteraciones de las paredes capilares

Recordamos al paso que mediante una brillante serie de investigaciones experimentales publicadas entre 1930 y 1934 Rocham y Nolf confirmaron los

Sayers, Sayer, Long y Long establecieron en 1946 que la corticotropina (ACTH) reduce el almacenamiento de la vitamina C en la corteza suprarrenal y Holly y McLe ter observaron en dos casos (1951) la aparición de manifestaciones de carencia de ácido ascórbico luego de prolongado tratamiento con corticotropina. En ambos casos ocurrió un síndrome de escorbuto: púrpura cutánea, gingivorragias, fragilidad capilar incrementada con protrombinemia, número de plaquetas, coagulación normales. La incorporación de vitamina C en altas dosis dominó rápidamente la situación, que recidivó a la nueva incorporación de corticotropina sola y que fué de nuevo dominada por vitamina C, la cual asociada a la corticotropina previno la aparición del síndrome escorbútico.

Huelga recordar al paso que con la incorporación de la vitamina C a la terapia —específica en la diátesis hemorrágica llamada escorbuto y producida por su carencia— se creyó resuelto el problema patogénico y terapéutico de las discrasias vásculosanguíneas hemorragíparas. Que dicha esperanza resultó fallida es hecho bien conocido. Con el descubrimiento en 1936 de la vitamina P, llamada de la permeabilidad vascular, por Szent Gyorgi, Armentano y Bensath, volvieron a abrigarse esperanzas de soluciones favorables muy parcialmente cumplidas, tanto con ella cuanto con el descubrimiento de Lavollay de que los derivados de las flavonas, señaladamente la catequina, incrementaban la resistencia vascular en los animales sometidos a regímenes escorbutígenos. Al restarse a todas estas sustancias el valor terapéutico absoluto que se les pretendió atribuir en un principio, no perdieron por ello todo su prestigio y su eficacia relativa, pero incontestable, los hace corrientemente usaderos en la práctica clínica. Y es que en las variadas discrasias vásculosanguíneas es por demás frecuente la deficiencia en vitamina C y asimismo de la vitamina K, en cuanto ellas se acompañan de una disfunción hepática. Macbeth y colaboradores destacan en 1943 estos hechos, confirmados por Neivert y colaboradores en 1948, en casos de epistaxis recidivantes comprobaron el descenso del tiempo de protrombina o de la cuantía de ácido ascórbico en la sangre en el 90 por ciento de los casos y el *descenso de ambos a la vez en el 33 por ciento de los casos.*

Y ya que de vitaminas y discrasias vásculosanguíneas estamos tratando procede recordar que H. S. Stannus, en 1948, al considerar los trastornos del sistema nervioso por alimentación deficiente, escribe que "existe también alguna evidencia que un trastorno del lecho capilar, *la disergia capilar*, es causado por una deficiencia de riboflavina coincidente con la afección del sistema nervioso".

La coexistencia de diátesis hemorrágicas con enfermedades infectivas agudas o crónicas es hecho viejo conocido de la clínica tradicional.

El antiguo concepto de la toxemia infectiva actuando directamente sobre la sangre, los vasos y si acaso el sistema nervioso, ha sido parcialmente destronado en los últimos decenios y reemplazado por el de la índole anafilacticoalérgica de la discrasia vásculosanguínea.

Decimos parcialmente destronado pues la observación clínica enseña que en *el decurso de ciertas enfermedades infectivas agudas o crónicas ocurren* episodios de púrpura que obedecen a dos mecanismos etiopatogénicos diferentes.

Concretándonos a la amigdalitis estreptocócica aguda como paradigma, hemos observado la púrpura apareciendo y cursando coetáneamente con ella y otras

Academia Nacional de Medicina, proponen nuevos procedimientos para esa estimación y lo propio hacen Knoll, Wilbrandt y Wyso en Suiza y Darnis en París.

Frouchtman y Llovera destacan en 1948 el frecuente incremento de la fragilidad capilar en las manifestaciones alérgicas, señaladamente en la urticaria.

El influjo menstrual sobre las manifestaciones hemorragíparas es conocido clínicamente de tiempo atrás, pero a pesar de las numerosas investigaciones llevadas a cabo el esclarecimiento de esa acción no se ha logrado aún. Nagy (1925) habló de purpura de ovárica. Minot (1936) trató la purpura menstrual, asunto prolijamente estudiado por A. Pavlovsky en publicaciones de 1951 a 1952.

El descenso premenstrual de las plaquetas y el incremento menstrual de la fragilidad vascular, señalados por diversos investigadores en los últimos veinte años, han sido incriminados en la génesis de *las hemorragias vicariantes* nasales, vesicales, pulmonares, cutáneas, etc., llamadas menstruales o catameniales.

Antenor Rey de Córdoba manifiesta que sus investigaciones no permiten atribuir a fragilidad capilar incrementada los fenómenos hemorrágicos catameniales.

Arnold, Holtz y Marx en 1936 logran en perros, mediante inyecciones de grandes dosis de hormonas gonádicas femeninas, la producción de purpura trombopénica.

A. Hernández Díaz en 1940 dedica un interesante trabajo a las *Hemoptisis neurovegetativas*, poniendo de relieve su vinculación endocrina en los comedios de la vida femenina, la menstruación, la gravidez y la menopausia, y asimismo con los influjos psíquicos y cósmicos.

En todas las manifestaciones hemorragíparas que aparenten tener alguna vinculación con las hormonas gonádicas debe siempre tenerse presente la hegemonía que sobre las endocrinas tiene el sistema nervioso central, punto tan destacado relieve por Stieve en su recientísima publicación de 1952.

Hirsch y Dameshek en su trabajo de 1951, basado en 89 casos de trombocitopenia idiopática, consignan una observación de purpura aparentemente inducida por estrogenoterapia, observación que parece pertenecer al intrincado y complejo asunto de la *alergia endocrina*, objeto de interesantes investigaciones del Prof. C. Ruiz Moreno y Dres. M. A. Solari y Fernández en el Departamento de Alergia del Instituto de Investigaciones de la Academia de Medicina durante los años 1950 a 1952.

Perranet y Bullet tratan en 1952 el complejo problema de los síndromes alérgicos y estados hormonales.

Al respecto corresponde recordar el caso publicado en 1950 por Dr. Atu. Zara, Solignac y Bocquin de purpura hemorrágica gravísima en una mujer, dominada merced a la incorporación de dosis masivas de testosterona.

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reacciones: la una *anafilácticoalérgica*, la otra, que llaman *Reacción Sanérgica*, que puede expresarse por dos procesos diferentes: el uno local y extenso, tipo Shwartzman, con lesiones cutáneas en el sitio de aplicación del sanérgico sensibilizante; el otro difuso, esencialmente hemorrágico, tipo Sanarelli, que reviste ora el tipo de la púrpura hemorrágica generalizada, *de manifestaciones hemorrágicas viscerales, de necrosis vasculares, de glomérulonefritis*, etc.

Estos novedosos y originales conceptos tienen incontestablemente una base clínica real, ya que son hechos de observación, sino corrientes, relativamente frecuentes en la práctica clínica, y que corresponde analizarlos prolijamente a fin de establecer si la *interpretación* fisiopatogénica de Lewis es o no exacta.

La alteración capilar en las reacciones anafilácticoalérgicas es clásica en la clínica; a ella se debe *la reacción exudativa* y la *extravasación sanguínea*. Se le atribuye a *histamina o sustancia tipo H de Lewis liberada en la reacción antígeno-anticuerpo*, la cual incrementaría a la vez o separadamente la permeabilidad y la fragilidad vascular. Sobre el particular volveremos muy luego.

El factor vascular ha interesado siempre en la hemofilia.

Virchow, en 1859, habló de anomalías en el sistema vascular; von Recklinghausen, en 1883, englobó a la hemofilia dentro de las diátesis hemorrágicas neuropáticas; en este siglo XX Sahli (1910), Abderhalden, Morawitz, Opitz, Wochlisch, Naegeli y otros le conceden importancia en la *génesis de las hemorragias, sobre todo espontáneas*. Schutz, en 1923, admite que en la hemofilia la falla principal radica en la función de los vasos. Schloessman, en su excelente publicación de 1930, piensa otro tanto y Hecht llega, en sus investigaciones publicadas en 1935, a admitir alteraciones periódicas y temporarias en la permeabilidad vascular de los hemofílicos. Fonio, en su importante trabajo de 1936, rechaza la alteración vascular en la hemofilia, que es admitido por Howell, en 1939, pues a pesar de la corrección en el tiempo de coagulación, las hemorragias subsisten.

A. Pavlovsky, en su importante trabajo "Consideraciones patogénicas y terapéuticas de la hemofilia", de 1946, laureado con el Premio M. R. Castex por la Academia Nacional de Medicina, escribe textualmente: "el factor vascular que a nuestro juicio interviene decididamente como factor morboso, a tal punto que hace dudar si una vez descubierto el recurso capaz de *modificar el alargamiento del tiempo de coagulación se habrá resuelto totalmente el problema del* tratamiento de la hemofilia".

A juicio de A. Pavlovsky, prescindiendo del trastorno de la coagulación exhaustivamente estudiado y evaluado por él, *el factor vascular interviene decididamente en el desencadenamiento de las crisis hemorrágicas*. Funda ésta su opinión en base a los hechos recogidos en su dilatada experiencia clínica personal, y la condensa en los siguientes términos:

1.°) A pesar de que el tiempo de coagulación se mantiene constantemente alargado, el *hemofílico puede pasar largos períodos sin sufrir de crisis hemorrágicas*. La aparición de éstas, a veces periódicas y hasta estacionales, otras sin causas justificadas o subsecuentemente a un primer trauma sin la adición de nuevos inductores, lo hacen creer en una alteración del factor vascular.

2.°) De la comparación de la hemofilia con la fibrinopenia congénita surgen hechos que abonan la intervención del factor vascular en la génesis de las crisis hemorrágicas del hemofílico. La hemofilia reviste mayor seriedad para la vida regular del aposentador que la *fibrinopenia congénita, a pesar de que en ésta el trastorno de coagulación es mucho más* grave, ya que la sangre no coagula y en la hemofilia lo hace siempre, aunque tardíamente.

veces la hemos visto aparecer subsecuentemente a ella. En esta segunda eventualidad la índole alérgica de la púrpura nos parece incontestable, así como nos parece también incontestable la índole toxicoinfectiva de la púrpura que aparece y cursa concomitantemente con la amigdalitis estreptocócica. Es lógico atribuir esta forma purpúrica a las toxinas estreptococicas, probablemente similares a la estreptoquinasa (fibrinolítica) y estreptodornasa (desoxirribonucleasa) que lisa nucleoproteínas) estudiadas por W. S. Tillet y Sherry en 1949 y ulteriormente *explotadas por ellos en el tratamiento de las pleuresías purulentas* y por Sherry, McCarty y Tillett (1951) en el tratamiento del absceso hepático amebiano, con cierto resultado, o del sistema hialuronidasa antihialuronidasa estreptocócica estudiado en 1952 por Caprio, Rantz y Randall, y vinculado principalmente con estreptococos hemolíticos del grupo A.

Hirsch y Dameshek, en su publicación de 1951 referente a 89 casos de trombocitopenia idiopática, mencionan con extenso fichero bibliográfico a las púrpuras por idiosincrasia o sensibilización a alimentos, drogas, enfermedades infectivas comunes agudas y exantemáticas.

Storck, Hoigne y Koller estudian en 1951 la patogenia de las diátesis hemorrágicas sobre *la base alérgica*. Admiten la etiogenia infectiva para gran número de las mismas. Conceden máxima importancia en la fisiopatogenia a la trombocitopenia, al incremento de la heparina antitrombina y a la ausencia del factor V. Admiten la ignorancia reinante en lo concerniente *al factor vascular* y piensan en base a sus investigaciones que *el grupo de las diátesis hemorrágicas vasculares puras*, que abarca entre otras afecciones la púrpura reumática, se irá reduciendo a medida que se perfeccionen los conocimientos acerca del mecanismo de la coagulación merced al refinamiento de los procedimientos modernos.

Criep y Cohen hacen en 1951 una revisión de conjunto sobre las publicaciones de *Púrpura como manifestación de alergia a la penicilina*, consignando tres observaciones propias, de cuyo estudio prolijo concluyen excluyendo el factor sanguíneo y responsabilizando al factor vascular en la génesis de las manifestaciones hemorrágicas.

Las publicaciones de Prigal (1948), Blue (1949), Kaufman (1949), Weiss y English (1949), French (1950) y Miller (1950), entre otros, evidencian que *los factores emocionales* modifican la extensión y la severidad de *las reacciones alérgicas*. U. Pipkorn, de Goteberg, sintetiza en 1952 el problema del psiquismo y alergia, concediendo capital importancia al sistema neurovegetativo y al trastorno de los mecanismos regulatorios de la personalidad armónica, que llama *disbiotonía*, y responsable del *síndrome disbiotónico*.

Lewis estudia en 1949 los síndromes clínicos por hipersensibilización a drogas, que designa con el nombre de *Síndrome quimioterápico secundario*.

Recuerda que el fenómeno de Sanarelli (1894) y el fenómeno de Shwartzman (19) son según Alechinsky (1939) fenómenos idénticos que no dependen de la alergia [illegible]. Introduce el concepto de *sanergia* (Sanarelli y ergia, reacción a drogas o t[illegible]rianas). La reacción de Sanarelli Shwartzman tendría por mediador principal el sistema nervioso autónomo, desconociéndose aún el mecanismo íntimo de esa mediación.

Boelter y Hatoff aplican este concepto en 1949 en el estudio de sujetos pr[illegible] que a la incorporación de drogas antibióticos, etc., reaccionan. Observan do[illegible]

en 1950 señaló el acortamiento de la coagulación en el ser humano bajo el efecto de la tensión nerviosa y Macht en 1952 comprobó lo propio en sujetos normales bajo el influjo de cargas emocionales, registrando un franco acortamiento de la coagulación en los sujetos aprehensivos y un marcadísimo acortamiento en los individuos muy impresionables.

El conocimiento de las adquisiciones de los últimos años sobre *anatomofisiología del sistema arteriolocapilar*, estudiados en revisiones de conjunto por H. Sánchez Caballero en 1950 en la Argentina y por Schiller en 1952 en los Estados Unidos, es indispensable, en nuestro sentir, para la justipreciación e interpretación de los fenómenos que se desarrollan en la pared capilar y que bajo forma de incremento de la permeabilidad o de la fragilidad ocurren y caracterizan a gran número de las diátesis hemorrágicas.

La permeabilidad capilar, función fisiológica consistente en el tránsito de los elementos plasmáticos, coloides y cristaloides a través de sus poros, está intrínsecamente relacionada con la morfología microscópica de la pared capilar integrada por tres componentes en continuidad, de los cuales la membrana semipermeable porosa es la trascendente, pues de sus cambios de porosidad depende la permeabilidad (Schiller 1952). La capa capilar porosa está constituida por las células endoteliales contiguas y la sustancia cementatoria circundante producto de las células endoteliales, según Chalmers y Zweifach (1940-1947). El cemento se ablanda y se hace más permeable cuando desciende el pH o cuando la cuantía de calcio cae debajo de un nivel crítico. El calcio incrementa la resistencia de la pared a la filtración, según Lasch y Kalond (1951). Las células endoteliales al contraerse individualmente bajo influjos mecánicos o químicos agrandan los poros del cemento, facilitando así la filtración y el tránsito de glóbulos rojos desde el interior del capilar.

Normalmente el cemento es de continuo erosionado por la corriente sanguínea y de continuo reemplazado mediante la elaboración por las células endoteliales. La fina capa de proteínas plasmáticas adsorbida por la superficie luminar, segunda componente de la pared capilar, así como también las plaquetas (Danielli 1940) gravitan asimismo sobre la porosidad de la sustancia cementatoria.

Entre los agentes capaces de incrementar esa porosidad, vale decir, la permeabilidad capilar, han sido precisados la hipoxia, la histamina o sustancia H, una de las dos fracciones activas que componen la hialuronidasa (Schiller 1952), la cual disgregaría, por su acción enzimática, la capa de polisacáridos de la pared interna capilar (Stans y Eckman 1951). Esta enzima hialuronidasa sería inhibida en su actividad por la vitamina C y la rutina.

La córticotropina y cortisona actuarían en los procesos inflamatorios agudos según Markin (1951) mediante la reducción de la permeabilidad capilar. Según otras investigaciones, los efectos periféricos de las hormonas adrenocorticales se deberían a una acción más dilatada, incidiendo sobre la sustancia fundamental del tejido conectivo y comprendiendo en su acción a la sustancia cementatoria, la vaina pericapilar y la reactividad de los elementos musculares lisos de los vasos terminales, las metarteriolas y los esfínteres precapilares.

La vaina pericapilar de sostén, especie de condensación del tejido fundamental

En la fibrinopenia congénita las manifestaciones hemorrágicas sólo ocurren bajo el influjo de fuertes traumas o intervenciones quirúrgicas, mientras que las hemorragias por pequeñas heridas se consiguen detener actuando exclusivamente sobre el factor vascular.

3) Los hemofílicos presentan crisis prolongadas, continuando sangrando a pesar del acortamiento y hasta de la normalización del tiempo de coagulación mediante transfusiones sanguíneas. Este hecho, observado reiteradas veces en hematurias de hemofílicos, y en un caso de hemorragia del frenillo del labio superior, en el cual, a pesar de la mejoría de la coagulación mediante transfusiones, fué menester ligar los vasos del frenillo para detener la hemorragia de una herida aparentemente trivial e insignificante por su pequeñez.

4) El desencadenamiento de crisis hemorrágicas por traumas emocionales. Ejemplo: dos hermanos hemofílicos al visitar al padre internado en un sanatorio subsecuentemente a un accidente, los cuales durante varios años en su niñez exhibieron crisis hemorrágicas en los días precedentes al festejo de sus cumpleaños, cargados de intensos influjos sensitivo-afectivos.

5) La cohibición de algunas crisis hemorrágicas por influjos psicológicos. De tal índole sería el influjo tenido por Rasputín sobre las hemorragias del zarevitz hemofílico. El influjo psíquico parecería determinar la vasoconstricción en la zona afectada, asiento probable de atonía capilar. Piensa A. Pavlovsky que durante las crisis hemorrágicas los hemofílicos sufren la pérdida del tonismo capilar fisiológico, impidiéndose así la detención de las hemorragias.

Que las cargas sensitivo-afectivas violentas, ya sean placenteras o displacenteras, repercuten sobre el sistema vasomotor con efectos variados, son hechos de observación cuotidiana en la práctica clínica.

La representación autónoma de la corteza prefrontal tiene el contralor supremo sobre los centros vegetativos hipotalámicos y, según se cree, por medio del haz neocórtico-septal, descubierto por Watts y Fulton.

Y si en el ser humano ocurre lo comprobado por Bender en 1938 en gatos y monos, que durante el miedo son vertidos dentro de la sangre acetilcolina y adrenalina, lo que implica la eventual estimulación orto o parasimpática durante la descarga emocional, se explican los trastornos vasomotores generales o zonales que pueden ocurrir, tanto en hemofílicos cuanto en los demás sujetos, expresando como hemorragias de origen emocional, cuales las ya mencionadas hematemesis por úlceras gastroduodenales observadas en Londres durante la blitzkrieg teutona en 1940. Y a este respecto referiremos brevemente una observación personal confirmatoria de lo que precede.

Padre e hijo, brasileros médicos, son atendidos por uno y otro de úlcera gastroduodenal con éxito aparentemente completo. Tiempo después la madre y abuela longeva fallece por angina de pecho en brazos del hijo médico, el cual sufre de inmediato una hematemesis copiosa que exige una transfusión de sangre; en el decurso de esta última, el segundo médico, hijo del primer médico y nieto de la finada, sufre de una hematemesis idéntica a la del padre, que impone los clásicos recursos de circunstancia. El trauma emocional en los dos colegas, padre e hijo, afectados por un mismo proceso aparentemente latente, indujo el mismísimo efecto: una copiosa *hematemesis*.

Corresponde asimismo de momento recordar que Cannon y Mendenhall comprobaron en animales la aceleración de la coagulación bajo el influjo del dolor y de la emoción; y que estos hechos experimentales en el animal, publicados en 1914, *han encontrado* su fenómeno gemelo en la especie humana, pues Schneider

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La vaina pericapilar de sostén, especie de condensación del tejido fundamental

conectivo intercelular, tercera componente de la pared capilar, gravita también sobre el transito de las sustancias desde el compartimento vascular

Chambers y Zweifach (1947) aplicando hialuronidasa sobre la superficie capilar mediante micropipetas, comprobaron la brusca aparición de hemorragias petequiales sin evidencia de aumento de la permeabilidad, admitiendo por ello *que la hialuronidasa y otros enzimas mucolíticos pueden ser factores que incrementan la fragilidad* más que la permeabilidad capilar

La fragilidad capilar es favorablemente influída por los flavonoides rutina, hesperidina citrina o *vitamina P o C que incrementan directa o indirectamente* el tono vasoconstrictor de los vasos terminales

La vitamina C es específicamente efectiva en la avitaminosis C (Schiller 1952) Investigaciones de Gabe y Parrot (1952) en cavias evidencian que sólo la asociación de la vitamina C con catequirra (vitamina P o C_2) previene el escorbuto y sus lesiones vasculares características La experiencia clínica demuestra la eficacia de la vitamina C y del zumo de limon fresco, en variadas discracias vasculares hemorragíparas

Macbeth informó en 1943 que las hemorragias operatorias y postoperatorias eran menos intensas en los casos que habían recibido un gramo de vitamina C durante los cuatro días que precedieron al tratamiento quirurgico de amígdalas senos o tabique

Neivert y colaboradores (1948) estimaron el ácido ascórbico y la protrombinemia en 101 casos de epistaxis en el 90 por ciento de los casos la cuantía de ácido ascorbico o el tiempo de protrombina eran anormales en un tercio de los casos ambos elementos se encontraban por debajo de la normal en el 45 por ciento de los casos el examen clínico no reveló la etiogenia de la epistaxis pero la incidencia de la anormalidad sanguínea en ácido ascórbico y tiempo de protrombina fué más o menos igual a la de los enfermos con causa local o general precisable El tratamiento con vitamina C y K benefició a 27 casos sobre un total de 36 enfermos

Stannus en 1948 consideró o la deficiencia de Riboflavina como causa de alteraciones simultáneas del sistema nervioso y del lecho capilar a cuyo nivel originaba la di ergia capilar

La administracion prolongada de corticotropina (ACTH) puede determinar la carencia de ácido ascorbico con el cuadro clínico clasico y completo del escorbuto *como lo evidencian las dos observaciones de Holly y Mc Lister de 1951* y asimismo la agravacion aguda letal de la esclerodermia cronica con produccion de lesiones fibrinoides y necroticas en las serosas sinovias articulaciones y glomérulos muy semejantes a las de la hipertonía maligna periarteritis nudosa y lupus eritematoso diseminado (Limseth Baker y Shifrin 1951)

Estos hechos por insolitos que sean constituyen una seria advertencia para el uso inadecuado de estas drogas modernas a menudo de efecto maravilloso cuando se las emplea en debida forma

Las hormonas adrenocorticales ejercen segun Robson y Duthie (1950) in flujo directo sobre la resistencia capilar efecto de la córticotropina explotado exitosamente *por Evans y Chi Kong Lin* (1951) en un caso de purpura cronico trombocitopénico

La circulacion arteriolocapilar esta adaptada a la actividad metabolica de las células para llevarles los nutrilitos y remover los catabolitos

La regulación de la circulación arteriolocapilar es llevada a cabo prevalentemente por mediadores químicos muchos de ellos productos del metabolismo celular ácido carbónico ácido láctico ácido adenílico calor, etc que originan la dilatación de los musculos lisos facilitándose así la remoción terminada ésta desaparece el estímulo vasodilatador y la circulación capilar recupera su ritmo precedente

La propiedad inherente de los musculos lisos de la metarteriola de los vasos directos proximales y de los esfínteres precapilares es la contracción rítmica o sea la contracción y relajación periódica llamada vasomotilidad *La actividad física* del musculo liso es inconstante está supeditada a la composición química del líquido ambiental y a la inervación Hecho importante *la vasomotilidad* de las metarteriolas y de los vasos directos puede estar disociada de la de los esfínteres precapilares

Los musculos lisos son exquisitamente sensibles al ambiente químico Zweifach observó que la epinefrina y los corticoesteroides estimulan la vasomotilidad esfinteriana y así incrementan la absorción los agentes bloqueadores adrenérgicos y algunos anestésicos inhiben la vasomotilidad esfinteriana

Jurgensen observó en 1920 que en *la parálisis vasomotora de la gripe* la microscopia capilar directa revelaba la dilatación y estancación en los capilares

Mediante igual procedimiento aplicado al lecho ungueal comprobó Davis en 1946 la frecuente incidencia de las petequias capilares en ausencia de purpura cutánea en estados de aterosclerosis purpura escorbuto bronquiectasias síndrome de Raynaud y de hipocratismo digital

Humble investiga el mecanismo de la formación de la petequia en 17 casos mediante la técnica Ultra Pak Leitz con brazal de compresión e informa (1949) que el sitio de la hemorragia esta localizado en el punto de union arteriola—gancho capilar o sea en el tramo arteriola—precapilar

La motilidad capilar aumenta en la estimulación simpatica, en la inyección de extractos corticosuprarrenales y de adrenalina y en la hemorragia aguda disminuye por el enfriamiento el ejercicio muscular los principios vasodepresores de la anoxia prolongada y las dosis elevadas de barbitúricos desaparece cuando se secciona el nervio correspondiente de la zona (Sánchez Caballero 1950)

Como prototipos de sustancias de acción vascular figuran *la adrenalina y la histamina* La adrenalina en relación a su concentración contrae la metarteriola el esfínter precapilar la porción proximal del canal central pero no contrae la porción distal de este ni la vénula amuscular ni los capilares

La palidez del tejido por concentraciones adecuadas de adrenalina se debe a la oclusión de los esfínteres precapilares y al escurrimiento de la sangre capilar hacia las vénulas La histamina en relación a su concentración dilata la metarteriola el esfínter precapilar la vena muscular del lecho capilar y lleva hasta la cesación completa de la motilidad los capilares llevan una corriente llena y continua no ejerce acción sobre los vasos amusculares (Sánchez Caballero 1950)

La vasomotilidad influye sobre la filtración o intercambio entre la sangre y los tejidos en forma directa determinando el camino que toma la sangre por el canal central o por los capilares verdaderos en forma indirecta por el influjo que

tiene el camino seguido sobre el caudal del escurrimiento venoso Las investigaciones de Chambers y Zweifach (1947) muestran que continua siendo cierto lo afirmado por Starling la filtracion depende de la relacion entre la presión hidrostatica y la presion coloidosmotica pero en realidad, quien rige la filtracion es *el aparato vasomotor* cuya actividad aumenta o disminuye la superficie sobre la cual la presión hidrostática efectiva podra producir una filtracion hacia afuera de los vasos (Sanchez Caballero 1950)

Al terminar esta breve síntesis sobre fisiologia de los capilares ponemos en destacado relieve la fundamental importancia que en ella tiene el sistema vasomotor ensanchando o reduciendo la superficie del lecho capilar

En las tres ultimas décadas se ha enriquecido la fisiologia normal y patologica del sistema vasomotor con el descubrimiento de *los baro y quemorreceptores arteriales* uno de los hechos de mas ejemplar gravitación en los trastornos vasculares en estudio Su conocimiento no puede menospreciarse y menos aun ignorarse ya que con repentinidad extraordinaria actuando en un todo homogéneo sin solución de continuidad en íntima interpenetración pueden modificar sectores circunscriptos o extensos del sistema vascular determinando perturbaciones circulatorias capaces de transformar las regiones circulatorias comprometidas en estructuras efectoras para las manifestaciones hemorragicas

Con los estudios de Hering (1923-1927) sobre los reflejos cardiovasculares a punto de arranque en *el seno carotídeo* y en *los nervios depresores de Cyon y aortico de Ludwig* conocidos desde el siglo XIX y con ulterioridad intensiva y proficuamente investigados por Hymans Bouckaert y Regniers (1933) se impuso la trascendencia de los reflejos originados en los baro y quemorreceptores vasculares cuyo conocimiento histologico debemos a las notables investigaciones de Fernando de Castro

Con posterioridad establece Latner que el seno carotídeo controla la medula osea y estimula por via refleja los centros cerebrales reguladores de la sangre

Quizás a este influjo se deba al menos en parte la hemorragia iterativa tardia en la herida operatoria accidental del seno carotideo tratada por sutura lateral publicada por De Ledesma en 1950

Anderson y colaboradores comprueban en 1950 que la excitacion de los quemorreceptores en las carotidas y aorta en las hemorragias es la causa de las ondas de Mayer en B P y de la respiración de Cheyne Stokes

Liljestrand senala en 1951 que la acetilcolina es el mediador químico del cuerpo carotídeo

Sucesivamente fueron revelados vasorreceptores en diversas arterias del cuerpo Se senalaron a nivel del tronco celiaco y de la arteria mesentérica superior (Heymans y Rombaux Farbert y Shu) de la arteria hepatica (Rein) de la arteria esplénica Waelle y van de Velde observaron que la excitacion eléctrica de la arteria esplénica elevaba la presion carotídea por reflejo vía vagal Scheiner logro idéntico efecto estimulando el nervio esplénico Tschermgencki variando las condiciones experimentales confirma y amplía esos resultados senalando la existencia de vasorreceptores esplénicos Mundo Fuentes (1947) investiga el reflejo presor originado en la compresión u oclusion de la arteria esplénica y

atribuye a la vasoconstricción refleja la súbita cohibición de la tendencia hemorragípara en la fase previa de la esplenectomía Holtz y Shumann señalan en 1950 la relación entre el seno carotídeo y la contracción esplénica

C Jiménez Díaz Barreda Paniagua y Molina (1947) comprueban que la excitación del cabo central del vago determina vasoconstricción seguida de hipertensión arterial y aceleración de la coagulación resultado que C Jiménez Díaz (1948) atribuye a la liberación de un fermento arterina por la pared arterial el cual actuaría sobre el plasma del animal reaccionando con el hipertensinógeno y dando origen a la arterohipertensina Insiste C Jiménez Díaz en 1951 sobre el particular manifestando que durante la estimulación del vago la pared arterial tanto en la circulación general cuanto en los territorios vasculares aislados libera noradrenalina a la cual se debe en parte prevalente la elevación de la presión sanguínea que dicha estimulación determina

Taylor Page y Corcoran invocan en 1951 un determinismo similar a nivel de las arterias encefálicas que llaman *mecanismo vasopresor hormonal neurogénico* para explicar *la hipertensión arterial de origen cerebral*

Page estudia en 1952 *el contralor humoral y vasomotor de los vasos sanguíneos* Admite que *las sustancias que controlan el calibre de los vasos* son de origen renal suprarrenal y neural y que las hay presoras y depresoras El *contralor de la reactividad de los vasos sanguíneos* está integrado por un sistema altamente complejo extensamente enraizado en todo el cuerpo en cuya regulación desempeñan importante papel *el sistema nervioso* (señaladamente sus ganglios autónomos los senos carotídeos los quemorreceptores cerebrales) *el hígado y el riñón* Es la interacción de fuerzas estimuladoras y depresoras actuantes sobre los vasos sanguíneos de responsividad cambiante lo que determina la circulación sanguínea y la presión

Como toque complementario y postreras consideraciones a todos los hechos clínicos anatomopatológicos y experimentales llevados a efecto en el ser humano y en el animal mencionados en esta ponencia nos aventuraremos a emitir una hipótesis sintética y holística acerca de la etiopatogenia de las diátesis hemorrágicas condensando nuestra opinión personal y en la que se coordina lo diverso y dispar en un tipo unitario de proceso a desenvolvimiento armónico

La etiofisiopatogenia de las diátesis hemorrágicas o discracias vasculosanguíneas descansa sobre un conjunto de procesos íntimamente conexionados que constituyen un todo homogéneo cuyos componentes unidos en seriación armónica contribuyen—prevaleciendo los unos sobre los otros según la forma clínica en juego—en la génesis del episodio hemorragíparo

La predisposición enraizada en la constitución individual constituye *el o los factores condicionantes*

La base constitutiva genotípica lleva en forma *congénita* y a menudo *hereditaria* los elementos de inferioridad que se expresan en el somatismo bajo forma de anomalías siempre parciales en los componentes del sistema hemovascular tales como *la hemofilia la fibrinopenia la telangiectasias de Rendu Osler* y demás *formas morbosas constitucionales*

Los más variados factores ecológicos ambientales traumáticos psíquicos infectivos tóxicos alimentarios etc gravitan sobre esa *base genotípica* por vía

de intoxicación sensibilizacion, carencia esforzamiento etcétera para crear *el paratipo* adicionado al genotipo

El terreno genoparatípico así originado en perpetua mudanza de reactividad lleva en si *el condicionalismo el prerrequisito* y es él quien ante agresiones de la índole mas variada, ante el vaivén *de coaliciones fugaces o iteradas decidira la* expresion clinica o sea que *la modelara* desempenando una *accion patoplastica estrictamente individual* razon por la cual causas idénticas podran exteriorizarse bajo formas clinicas distintas en consonancia con la base genoparatipica y que causas dispares pueden engendrar un efecto idéntico actuando sobre un mismo sujeto Siendo cada uno de estos dos factores mencionados deficientes por sí para engendrar la enfermedad requieren el auxilio recíproco o sea la concurrencia dual para crear la afeccion que así resulta producto y consecuencia de esta interaccion combinada

Los factores determinantes de la afección hemorragípara o desencadenantes de los episodios agudos varian considerablemente tanto en su esencia cuanto en su procedencia constituyendo a veces verdaderas ristras de causas y concausas Pueden desplegar una *accion circunscrita* (influjo físico o mecanico por ejemplo) *o difusa con repercusion general* traumatismos emociones exo y endotoxicosis infecciones agudas o crónicas alimentacion viciada o deficiente etc pudiendo muchas de ellas actuar *por vía general* de toxemia sensibilizacion avitaminosis etc

El impacto de este factor provocador o generador puede hacerse *directamente* sobre el *sistema hemopoyetico en su totalidad* (anemia aplastica panmielotisis) o sobre *una cualquiera de sus componentes* (agranulocitosis trombocitopenia etc) o sobre *el sistema vascular* en forma regional o difusa o puede hacerse *indirectamente por vía del sistema nervioso central* injuriando parcial o totalmente *los centros trofodinamicos* que regulan *la actividad vascular* o los que regulan *el sistema hematopoyético en su integrantes celulares y liquidos*

Haran de *estructuras efectoras o responsivas* aquéllas condicionadas en el genoparatipo vale decir una vez más que éste modela patoplasticamente el efecto de la agresion

En armonía con la acción de la causa unica iterada o sostenida violenta o leve sera la expresion clinica que revelara en el somatismo *la accion periférica vascular o sanguínea o la accion central nerviosa* cuyos *centros repetimos regulan la composicion sanguínea* en sus integrantes celulares y liquidos y *la funcion vascular* señaladamente en su componente vasomotor bajo forma de trastornos generalizados o extensos o regionales a distribución bilateral simetrica radicular o periférica en consonancia con el sitio del sistema nervioso vasomotor agredido por la causa morbosa

La agresion directa o indirecta por *vía del sistema nervioso puede manifestar* e por la parestaeohibicion o por la paralisisinhibicion funcional de la angre *in toto* o de cualquiera de sus integrantes o del sistema vascular

La disfuncion vascular así originada lesional directa difusa o zonal o funcional neurogénica—que puede a través de la estancación originar la hemorragia por diapédesis experimentalmente lograda y en su subsistencia llegar a hacer e

lesional con la sección fisiológica primero y anatómica luego del nervio vascular —determina trastornos en la actividad vasculocapilar, cuyos mecanismos quedaron ya señalados

La alteración de la capa del endoteliocemento o de revestimiento interno se expresarán por anormalidades de la permeabilidad, la de la hoja pericapilar por anormalidades en la resistencia capilar con incremento de la fragilidad capilar sufriendo por ello la filtración o intercambio entre sangre y tejidos ya que ella está regida señaladamente por el aparato de la vasomotilidad

En cualquiera de los momentos de esta larga cadena de procesos pueden intervenir *reflejos vasomotores adicionales* a punto de arranque en los *múltiples baro y quemorreceptores arteriales* mencionados anteriormente

Esta sinopsis etiopatogenética general de las diatesis hemorrágicas permite interpretar plausiblemente los *estados hemorragíparos agudos y crónicos* con componentes dominantes o exclusivos del sector vascular o del sector hemático parcial o totalmente afectado (trombopenias leucosis panmielotisis) y así mismo *los episodios hemorragíparos agudos* en las infecciones agudas o crónicas en las intoxicaciones agudas o crónicas en las avitaminosis en las endocrinopatías en las discracias agudas o crónicas de origen visceral renal hepático cardíal etc *y de los estados morbosos vasculosanguíneos permanentes o crónicos* como ocurre por ejemplo 1) en la *trombopenia esencial crónica* en que a veces basta la leve frotación cutánea para provocar la purpura 2) *en la hemofilia*, en las *hemorragias espontáneas* que suelen subsistir pese a la normalización del tiempo de coagulación mediante transfusiones sanguíneas para explicar las cuales debe buscarse *el factor adicional generador* siempre presente de índole vascular o neurovascular a causa variada una ajeno a la anomalía hereditaria y congénita de la coagulación sanguínea

En el cuerpo humano unidad anatómica y verdadera unidad funcional no se manifiesta entre los varios órganos y estructuras relación de inferioridad y superioridad sino de solidaridad de funciones que forman un todo homogéneo sin solución de continuidad pero cuyo tono reaccionario está en perpetua mudanza siendo de todo punto imposible que dejen de actuar convergentemente de consuno las influencias morbígenas dependiendo de la base genoparatípica la expresión clínica así como los atributos adscriptos a cada fenómeno surgido en el somatismo

SUMARIO

Considera los diversos factores que intervienen en la hemostasia la formación del trombo la adhesividad de los endotelios la función de prevención la contracción vascular activa y las reacciones vasculares a distancia

Seguidamente pone de relieve los dos factores que intervienen en las hemorragias en las discrasias vásculo sanguíneas el factor sanguíneo y el factor vascular

Aborda el estudio del influjo trofodinámico del sistema nervioso sobre los vasos sanguíneos iniciándolo por una breve revisión histórica de la cuestión Analiza las observaciones clínicas los hechos anátomo patológicos y las investigaciones experimentales que evidencian la existencia de las hemorragias de origen neural Estudia el sistema nervioso vasomotor la importancia de su integridad anatómica y funcional la resistencia o fragilidad vascular el influjo endocrino gonádico suprarrenal e hipofisario Considera las discra

sias vásculo-sanguíneas de origen toxiinfectivo y anafiláctico alérgico Destaca el factor vascular en la hemofilia y pone de relieve las investigaciones de Alfredo Pavlovsky Estudia el influjo psíquico Se detiene en la anátomo fisiología del sistema capilar su integración la motilidad la estructura las funciones de sus componentes señaladamente sobre la permeabilidad y la fragilidad capilar

Pasa rápidamente *revista a los vasos y quimioreceptores destacando la importancia de* las reacciones arteriales reflejas Termina exponiendo en forma sintética el concepto personal sobre la etiofisiopatogenia de la diátesis hemorrágicas

THE VASCULAR FACTOR IN HEMOSTASIS AND HEMORRHAGE

The diverse factors that intervene in hemostasis (thrombus formation the adhesiveness of the endothelium the prevention function the active vascular contraction and the later vascular reactions) are considered

Subsequently emphasis is placed on the two factors that intervene in the hemorrhages of the vascular blood dyscrasias the blood factor and the vascular factor

The study of the tropho-dynamic influence of the nervous system on the blood vessel is entered upon starting with a brief historical review of the question The clinical observations the anatomico pathological facts and experimental investigations that show the existence of hemorrhages of neural origin are analyzed The vasomotor nervous system the importance of its anatomic and functional integrity the vascular resistance or fragility the endocrine gonad adrenal and hypophysial influence are studied The vasculo-blood dyscrasias of toxi infectious and allergic anaphylactic origin are considered The vascular factor in hemophilia is stressed and Alfredo Pavlovsky's investigations are enhanced The psychic influence is studied The author stresses the anatomico-physiology of the capillary system its constituents the motility its structure the functions of its components notably upon its permeability and capillar fragility

A brief review is made of the vessels and chemoreceptors pointing out the importance of the reflex arterial reactions The author ends by expounding in synthesis a personal concept on the etiopathology of hemorrhagic diathesis

I-2

Papel de la Hipófisis sobre la Sangre Hemocitopoyesis y Tejido Linfoide

B A HOUSSAY *

LAS HORMONAS *no inician procesos* metabólicos o funcionales nuevos pero pueden regular o modificar los existentes Los procesos de formación y destrucción de las células de la sangre y de los tejidos linfoide y mieloide se producen en ausencia de las glándulas de secreción interna Pero estas por medio de sus hormonas ejercen una acción reguladora sobre los procesos de metabolismo y crecimiento del organismo de los vertebrados y por ellos intervienen en la homeostasis de los elementos de la sangre médula ósea y tejido linfoide etc —La falta o el exceso de algunas hormonas sean endógenas o exógenas puede provocar modificaciones en la sangre y en dichos tejidos

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La *pars distalis* de la hipófisis, órgano central de la constelación endocrina desempeña un papel regulador importante sobre la sangre y los tejidos hemocitopoyéticos, que en parte es directo (somatotrofina) y en parte indirecto (a través de la tiroides, gónadas y suprarrenal).

Las modificaciones sanguíneas producidas por la insuficiencia hipofisaria son tanto mayores cuanto más se afecta el crecimiento o más intensos son los trastornos metabólicos. Por ello son bien manifiestas en la rata, el hombre y los perros cachorros y muy escasas en los perros adultos.

En los perros cachorros hipofisoprivos (1 de Aschner 1912, 14 nuestros estudiados, 11 por Conalons 1916 y 3 por Parodi 1938) se ha observado: 1) paro o retraso del crecimiento corporal; b) menor concentración de eritrocitos (−18%) que en los hermanos sanos; c) menor concentración de reticulocitos (pocas observaciones); d) ligera leucocitosis (+16%) aunque no siempre (9/14); e) generalmente una eosinofilia marcada (+80%); f) eritrosedimentación más rápida.

En 33 perros adultos hipofisoprivos que he operado se comprobó primeramente una disminución de hemoglobina y glóbulos rojos (Houssay, Royer y Orias 1931, Varela 1932, Parodi 1934) pero más tarde se encontró que esa disminución era pasajera (Parodi 1938–40). En cambio era constante el aumento de resistencia globular (Parodi 1938). Primero se halló leucocitosis con neutrofilia (Varela 1932) pero que fué pasajera, volviéndose a una concentración normal un poco baja de leucocitos; el hemograma de Schilling presentaba desviación a la derecha (Parodi 1937–40). Se halló una eosinofilia marcada (Varela 1932, Parodi 1938–40). Los neutrófilos fagocitaban menos que los de los perros normales (Parodi 1934–35–40). Los perros hipofisoprivos formaban más antitoxina diftérica (Savino 1935) e igual aglutinina tífica (Ferrer Zanchi, 1936) que los perros testigos normales.

La regeneración sanguínea después de una sangría fué igual que en los normales, pero la reticulocitosis fué mucho más intensa y prolongada en los perros hipofisoprivos, aunque era igual su concentración de reticulocitos antes de la sangría (Varela 1932).

La hipofisectomía produce en la rata modificaciones sanguíneas mucho más profundas (Meyer y col. 1937, Vollmer y col. 1939–41, Crafts 1941 a 1952, Gordon y Charipper 1947, Arvy y col. 1948, etc.). Ellas son disminución de la concentración de eritrocitos (un 30% o más) del volumen en el hematocrito (33%) y la hemoglobina (31%), fuerte disminución de reticulocitos. La anemia es ligeramente microcítica e hipocrómica. El volumen de eritrocitos de toda la sangre disminuye un 45% (Berlin y col. 1950, García y col. 1951). Está aumentada la resistencia de los eritrocitos a las soluciones hipotónicas (Arvy y col. 1948, Gordon y Megel 1951). En la médula ósea hay una hipoplasia medular global de intensidad moderada (Arvy y col. 1948) con disminución marcada de la eritropoyesis (Stewart y col. 1935, Meyer y col. 1936, Querido y Overbeek 1938, Vollmer y col. 1939–42, Crafts 1946–49, etc.).

Los leucocitos están en concentración normal o bien aumentados con linfocitosis y alguna disminución de granulocitos. Los eosinófilos suben durante los

2 a 9 días después de la operacion (R Houssay, 1951) La concentracion de plaquetas es baja y su ascenso es subnormal después de la esplenectomía (Adams 1949)

La respuesta a la hipoxia no se observa a 422 mm Hg de presión como en los normales (Stewart y col 1935 Meyer y col 1936–40), pero se produce normal mente si la presión de oxígeno es menor (322 mm Hg Feigin y Gordon 1950) Despues de la fenilhidrazina (Querido y Overbeek, 1938) y la sangria (Finkel stein y col 1944) se observa una buena regeneracion sanguínea reticulocitosis y estímulo de la médula osea Esta es menos sensible, pero capaz de reaccionar

La reticulopenia es mejorada por numerosas hormonas hipofisarias Los eritrocitos pueden aumentar por el extracto total de la hipofisis adreno corticotrofina gonadotrofina (en machos) y tirotrofina Los mejores resultados se han obtenido asociando una dieta rica en proteina con inyecciones de tiroxina y testosterona lo que normaliza la sangre y previene la anemia (Crafts 1949) Se obtienen mejorías marcadas de los eritrocitos y la médula osea pero *no completa* de la hemoglobina dando por separado tiroxina o testosterona con hierro El cobalto puede evitar la anemia y aun provocar en la rata hipofisoprıva una policitemia mayor que en las normales su accion supera la de cualquier otro tratamiento en la rata hopofisopriva (Crafts 1952)

En la insuficiencia hipofisaria humana es muy frecuente la anemia tanto en la caquexia hipofisaria de Simmonds como en el síndrome de Sheehan de necrosis de la hipófisis post partum y sin caquexia ni hiponutricion Puede comprobarse ésto en las recopilaciones casuisticas de Graubner (1925) Calder (1932) Silver (1933) Sheehan (1939) y en la muy completa de Escamilla y Lisser (1942) Estos hallaron sobre 57 casos verificados una media de 65 % de hemoglobina 3 7 millones de globulos rojos y 6 3 % de eosinofilos Podesta y Precerutti revi saron 22 casos publicados recientemente y hallaron que había anemia en 18 % (1 con menos de 2 millones de eritrocitos por mm³ 3 con 2 a 3 10 con 3 a 4 y 5 con más de 4) de 23 casos hubo leucopenia ligera en la mitad (5 a 10 000 leuco citos por mm³ en 10 casos o sea 43 4 % valores normales en 11 (47 8 %) y aumen tados en 2 (8 6 %) Se han publicado algunos casos de anemias muy intensas

Sheehan (1949) ha reunido 80 casos de la literatura o propios y ha observado que en general se instala progresivamente una anemia que a los 10 anos es de 3 a 4 millones de eritrocitos y luego persiste a ese nivel o desciende a 3 5—2 millones Examino 80 casos de la literatura y encontro en media 3 8 millones de eritrocitos 70 % de Hb 6 600 leucocitos 56 % de neutrofilos 3 5 % de eosinófilos 0 1 % de basofilos 37 2 % de linfocitos y 2 9 % de monocitos

La anemia es normocromica (Sheehan y Summers 1949) pero hubo casos de macrocítica hopocromica de normocítica hipocromica algunos de hipercromica pocos de anemia aplástica (Bloom y Bryson 1948) y anemia perniciosa (Watts 1932) Los leucocitos están en cantidad normal o un poco baja La linfocitosis relativa es frecuente pero no la absoluta En 1939 senala que la eosinofilia es comun (1/3 de casos entre 7 y 12 1/3 entre 3 y 6 y 1/3 menos de 2) pero en 1949 dice que existe a veces

El tratamiento de la anemia no es aun satisfactorio pero las medicaciones mas utiles fueron la testosterona y la cortisona (pocos casos estudiados) poca accion

de la tiroides y no mejoraron con hierro (Sheehan y Summers 1949-1952). Sheehan ha hecho notar que la causa más común de la insuficiencia hipofisaria es la necrosis post partum y que no suele acompañarse generalmente de caquexia como se creía desde Simmonds y aceptaban Escamilla y Lisser (1942).

En las modificaciones sanguíneas post hipofisectomía además de la supresión de las hormonas hipofisarias interviene la influencia agravante de la anorexia e hiponutrición sobre todo en la rata hipofisopriva y en la caquexia hipofisaria humana, pero no existe en el síndrome de Sheehan.

La anemia de los hipofisoprivos no se debe exclusivamente a la hipofunción tiroidea o testicular, puesto que es más intensa que la que producen la castración del macho o la tiroidectomía y además se observa en la hembra. La hormona de crecimiento aumenta los reticulocitos de los machos hipofisoprivos o castrados.

La acción hipofisaria sobre la hemocitopoyesis puede ser a la vez directa (hormona de crecimiento) e indirecta (a través de la tiroides, testículo y suprarrenal). La anemia hipofisopriva ha sido atribuída por unos a exceso de destrucción y por otros a disminución de la producción de eritrocitos y hemoglobinas. El punto no está dilucidado, pero hablan en favor de la menor formación: a) la reticulopenia; b) la marcada hipoplasia de la médula ósea y su menor reacción a la hipoxia; c) la disminución del crecimiento general.

La hipófisis parece estimular la eritropoyesis por su acción estimulante sobre el crecimiento celular y el anabolismo porteico que se ejerce sobre todo el organismo y por lo tanto sobre el tejido eritropoyético. En los cachorros y ratas hipofisoprivas hay retraso del crecimiento y a la vez anemia, mientras que en los perros adultos no hay ya crecimiento y la anemia es transitoria e inconstante.

Hay un paralelismo en la insuficiencia hipofisaria entre el retraso de crecimiento, la escasa formación de proteína y la disminución de la eritropoyesis.

La prevención de la anemia de la rata hipofisopriva se ha obtenido asociando un régimen rico en proteínas con testosterona y tiroxina, hormonas que aumentan el anabolismo porteico. El mecanismo de acción de estas hormonas y su punto de ataque no se conoce aún.

En el sapo *Bufo arenarum* Hensel la hipofisectomía produce un trastorno de distribución sanguínea. Se observa marcada dilatación de las arteriolas, capilares y vénulas, en los cuales se acumulan los eritrocitos, cuya concentración disminuye en el corazón y aorta, pero su masa total no varía (Aubrun 1935, Parodi 1937). Hay leucopenia, neutropenia y eosinopenia sin variación de los linfocitos (Varela y Sellares 1934).

La inyección repetida de extracto de lóbulo posterior produce una anemia intensa en el conejo (Dodds y Noble 1935-1937), la cual parece deberse a que produce oliguria, hemodilución y hemólisis (Gilman y Goodman 1935-1939). Con dosis menores puede provocar policitemias transitorias (Davis 1942). El extracto de lóbulo anterior contiene substancias que disminuyen (Ruitinga) y que aumentan los eritrocitos (Flaks y col.).

Algunas lesiones patológicas o experimentales del diencéfalo pueden provocar poliglobulias o leucocitosis. Su mecanismo es desconocido, así como la posible participación de la hipófisis en esos casos.

El bazo de los perros cachorros pesa menos que el de sus hermanos testigos

normales, su peso guarda relacion con el peso corporal o es menor (Ascoli y Legnani 1912 Houssay y Lascano González 1934) En los adultos no se halló diferencia entre el peso del bazo de 48 hipofisoprivos y de 68 testigos (Houssay y Lascano González 1934) Tanto en los perros como en las ratas hipofisoprivas está muy aumentada la pulpa blanca Los folículos son mas densos tienen menor diametro en los cachorros pero son mas voluminosos en los perros adultos con mayor diferenciación de sus tres zonas (Houssay y Lascano González 1934)

En la rata hipofisopriva el bazo se atrofia a la mitad en un mes este descenso de peso es proporcionalmente más del doble que el del peso corporal (Smith 1930) Perla 1936 Houssay 1947) pero no es tan grande si se practica la alimentacion forzada (H Houssay 1941–47) El tejido conjuntivo es mas aparente hay mas folículos por unidad de superficie y los centros germinativos son anchos y activos (Arvy y col , 1948) La atrofia del bazo por hipofisectomía se ha observado tambien en el conejo (White 1933) y el gato (McPhail 1935)

El extracto alcalino de hipofisis (Perla 1936) y un extracto rico en hormona de crecimiento (Marx y col 1941) hipertrofian al bazo En la acromegalia es muy frecuente el agrandamiento del bazo

La *pars distalis* de la hipófisis es uno de los factores fisiologicos que ejerce *una accion reguladora continua sobre los órganos linfoides y la hemocitopoyesis y* homeostasis de los globulos blancos sanguíneos Por su accion sobre las glandulas endocrinas que segregan hormonas esteroides corticoadrenal testículo y ovario ejerce una accion frenadora o moderadora sobre el desarrollo del tejido linfoide Por intermedio de otras hormonas somatotrofina y tirotrofina, ejerce una acción estimulante sobre el mismo La influencia de estos factores es mas intensa sobre el timo luego sobre el tejido linfoide difuso es menos intensa sobre los ganglios y mucho menos aun sobre el bazo

Después de la hipofisectomía suele observarse en general pero no siempre una involucion precoz del timo Esto se ha observado en 19 perros cachorros hipofisoprivos (9 familias) operados por mí (Houssay y Lascano González 1934 H Houssay 1947) también se ha observado en conejos (Saito 1934) En la rata hipofisopriva disminuye de peso (Smith 1930 H Houssay 1947) aun cuando por alimentación forzada suba el peso corporal la implantacion diaria de hipófisis de rata hace aumentar el timo de la rata sin hipófisis (H Houssay 1947)

Las gonadas y la corticoadrenal ejercen una influencia moderada constante sobre el timo pero no desempenan ningun papel en su involucion fisiológica La castración (Chiodi 1948) y la adrenalectomía (Rapela 1944) producen una hipertrofia del timo La inyeccion de gonadotrofinas produce involución del timo pero no en los castrados La adrenocorticotrofina exogena o endogena provoca una involucion del timo pero no en los suprarrenoprivo en estos la producen el extracto o las hormonas corticoadrenales La atrofia del timo (involucion accidental) de la reaccion de alarma se debe casi siempre a la accion hipofiso adrenal (hipersecrecion de adrenocorticotrofina que a su vez aumenta la secrecion corticoadrenal) (Selye 1950–51) Pocos agentes producen directamente la involucion del timo en ausencia de las suprarrenales (rayos X mostaza de nitrogeno)

En la acromegalia es bastante frecuente hallar un timo de tamaño supranormal En los animales hipofisoprivos hay poca modificación de los ganglios pero con más frecuencia se ha observado alguna hiperplasia linfoide La hipoalimentación obrando a través de la suprarrenal produce involución intensa del timo

La adrenocorticotrofina ejerce una acción moderadora continua sobre el tejido linfoide, por intermedio de la cortico adrenal La inyección de adreno córticotrofina produce una linfolisis intensa con disolución abundante de linfocitos e hipertrofia del tejido reticular en especial en el timo Los órganos linfáticos disminuyen de volumen Estos fenómenos son pasajeros y no se observan en ausencia de la suprarrenal (Dougherty y White 1943–47)

En la sangre se observa por inyección de la adrenocorticotrofina o en la reacción de alarma fenómenos iniciales y secundarios Los iniciales de corta duración consisten en un aumento de neutrófilos linfocitos y eosinófilos Los secundarios son más característicos y prolongados, consisten en linfopenia (Dougherty y White 1943–47) y eosinopenia (Dalton y Selye Hills y col 1948) La linfopenia y la eosinopenia no se observan en ausencia de la suprarrenal pero sin ella persisten muy intensas las variaciones iniciales (leucocitosis neutrófila eosinofilia y a menudo linfocitosis)

En el hombre es mucho más intensa característica y constante la eosinopenia que la linfopenia (Sprague 1951) Por eso se emplea preferentemente la primera como test de descarga de hormonas cortico adrenales

En la enfermedad de Cushing se ha observado un aumento de leucocitos y de neutrófilos con linfopenia relativa y en general absoluta la linfopenia persistente apoyaría el diagnóstico de ese síndrome (De la Balze Reifenstein y Albright 1946) Aunque la existencia de la linfopenia ha sido confirmada en muchos casos no es constante y su ausencia no invalida el diagnostico de enfermedad de Cushing o hipercorticalismo por tumor suprarrenal (Cervino y col 1949 Sprague 1951)

Al disolverse los linfocitos se liberan globulinas (β γ) Se ha afirmado que aumentan los anticuerpos pues éstos estarían almacenados especialmente en los linfocitos (Dougherty Chase y White, 1944–48) pero esto último es discutido y no puede considerarse demostrado

La regresión del tejido linfoide patológico por acción de la adrenocorticotrofina o cortisona es incompleta y muy pasajera en las leucemias linfosarcomas y en diversas adenopatías o esplenopatías malignas

La adrenocorticotrofina puede provocar algún aumento de reticulocitos y eritropoyesis en algunos casos de anemia

Numerosos estímulos accidentales intensos (stress) provocan descargas de adrenocórticotrofina y esta produce hipersecreción de hormonas adrenocorticales que causan una eosinopenia en la sangre Dichos estímulos no producen la eosinopenia en ausencia de la suprarrenal y falta también generalmente en ausencia de la antero hipófisis La acción de los estímulos sobre la hipófisis puede hacerse por doble vía directa o bien indirecta (por intermedio del sistema nervioso y acción hipotálamo hipofisaria) En el líquido peritoneal no se observa la disminución de los eosinófilos que se observa en la sangre durante la activación hipofiso adrenal o por inyección de corticoides (Speirs 1951)

La eosinopenia producida por la adrenocorticotrofina depende siempre de la activacion funcional de la suprarrenal, nunca se observa en los sujetos adrenalectomizados o en addisonianos (Thorn) Es mejor inyectarla por via intravenosa (25 a 30 mg en 8 horas en solucion de glucosa o salina) La reaccion mas sensible es la eosinopenia luego la excrecion urinaria de 11 oxi esteroides y en tercer lugar el aumento de 17 cetoesteroides (Thorn)

La adrenalina a la dosis de 0 3 mg se emplea como estimulante de la secrecion de adrenocorticotrofina, la cual a su vez produce eosinofilopenia Pero no es una prueba específica de exploracion de la función suprarrenal En efecto con 0 5 mg puede provocar eosinopenia en algunos addisonianos y la ha provocado en sujetos a los que se le habían extirpado las dos suprarrenales (Thorn) Debe existir otro mecanismo que produce eosinopenia por acción de la adrenalina sin intervencion suprarrenal

La eosinopenia producida por la adrenocorticotrofina depende de la respuesta suprarrenal y tambien de los mecanismos periféricos que producen la disminucion de eosinofilos La eosinopenia provocada por estímulos intensos (stress) depende de la respuesta de (a) ciertos centros nerviosos (hipotalamo) (b) de la antero hipofisis (c) de la corteza suprarrenal (d) de los mecanismos periféricos Por estas razones la presencia o falta de eosinopenia no es un índice absoluto del estado funcional suprarrenal aunque puede ser una prueba diagnóstica valiosa del mismo Tampoco se puede afirmar que esa prueba explora todas las funciones suprarrenales

La hipofisis produce acciones reguladoras sobre diversas glándulas endocrinas y sobre las funciones de metabolismo y crecimiento las ejerce también sobre la hemocitopoyesis el tejido linfoide y la homeostasis sanguínea en el estado normal y en condiciones patologicas (stress) etc)

Resumen

Las hormonas ejercen una accion reguladora sobre los procesos del metabolismo y crecimiento de este modo intervienen en la homeostasis de los elementos de la sangre médula ósea tejido linfoide etc

La *pars distalis* de la hipófisis actua en parte directamente (somatotrofina) en parte indirectamente (a través de otras glándulas) sobre la sangre y tejidos hemocitopoyéticos

Las modificaciones sanguíneas debidas a insuficiencia hipofisaria son más manifiestas cuanto más se afecta el crecimiento o más intensos son los trastornos metabólicos Por ello se manifiesta más en la rata hombre y perros cachorros que en los perros adultos

En los cachorros hipófisoprivos se observa aparte del retraso de crecimiento menor concentracion de eritrocitos y reticulocitos ligera leucocitosis eosinofilia y eritrosedimentación más rápida y modificaciones semejantes pero transitorias se encuentran tambien en perros adultos En la rata la hipofisectomía produce trastornos semejantes pero más marcados

Las alteraciones de los hematíes (anemia reticulopenia) pueden combatir e con extracto total de hipofisis y diversas tropinas u otras hormonas (el mejor resultado obtiene con dieta rica en proteinas e inyección de tiroxina y testosterona) El cobalto puede evitar la anemia y hasta provocar en las ratas hipófisoprivas mayor policitemia que en las normales *En la insuficiencia hipofisaria* humana se observa anemia de ordinario normocrómica tendencia a la leucopenia con neutropenia y en ocasiones eosinofilia La medicación mas útil parece ser la testosterona y la cortisona

La anemia de los hipófisoprivos no se debe exclusivamente a la hipofunción tiroidea o testicular (acción indirecta) pues es más intensa que la que produce la tiroidectomía o la castracion del macho (intervención de la acción directa por la hormona de crecimiento)

Lsa anemia ha sido atribuida a exceso de destruccion o a disminución de la formación de eritrocitos aunque parece que se debe a esta ultima causa La hipofisis excita la eritropoyesis por su acción estimulante sobre el crecimiento celular y el anabolismo proteico

La inyección repetida de extracto del lóbulo posterior produce en el conejo anemia intensa debido a oliguria hemodilución y hemólisis Dosis menores pueden provocar policitemia que también se observa después de lesiones patológicas o experimentales del diencéfalo

En los perros cachorros pero no en los adultos así como en la rata y conejo la hipofisectomía disminuye el peso del bazo con aumento de la pulpa blanca El extracto alcohólico de la hipofisis y el extracto rico en hormona de crecimiento hipertrofia el bazo

La *pars distalis* de la hipófisis ejerce accion reguladora sobre los órganos linfoides Por su acción sobre glándulas que segregan hormonas esteroides frenan su desarrollo en cambio la somatotrofina y la tirotrofina lo estimulan La influencia de aquel factor es más intensa sobre el timo menos sobre el tejido linfoide difuso aun menos sobre los ganglios y mucho menos sobre el bazo

La inyección de adrenocórticotrofina produce en la sangre fenomenos iniciales aumento de neutrófilos linfocitos y eosinófilos y secundarios mas característicos linfopenia y eosinopenia Esta última se utiliza como test de la descarga de hormonas corticoadrenales La eosinopenia producida por la corticotrofina depende pues de la respuesta suprarrenal pero también de mecanismos periféricos De todos modos aunque no sea un índice absoluto del estado funcional suprarrenal es una prueba diagnóstica valiosa

La inyección de adrenalina no es una prueba específica en la exploración de la función suprarrenal y deben existir otros mecanismos que producen eosinopenia

Action of the Hypophysis on the Blood on Hemopoiesis and Lymphoid Tissue

The hormones have a regulating action on growth and the metabolic processes They play a role in the homeostasis of the blood elements on the bone marrow on the lymphatic tissues etc

The pars distalis of the hypophysis governs the blood and hemopoietic system by two mechanisms one is direct through it s somatotrophine and in an indirect way through other glands such as the thyroid gonads and adrenals

Insufficiency of the hypophysis causes the blood to undergo modification the importance of which is in direct relationship to the normal development of growth and the intensity of metabolic troubles Thus they are clearly manifest and apparent in rats in human beings and in puppies whereas they are scarcely noticeable in adult dogs

In hypophysectomized puppies one finds apart from the delay in body growth a lower concentration of erythrocytes and reticulocytes slight leucocytosis eosinophylia and increased sedimentation rate Similar but transient modifications are found in adult dogs In rats hypophysectomy produces similar changes but more marked

The alterations of the erythrocytes (anemia reticulopenia) can be treated with total extracts of hypophysis and different trophines or other hormones such as thyroxin and testosterone together with diets rich in proteins Cobalt may avoid anemia and may even provoke in hypophysectomized rats a polycythemia superior to that observed in normal rats

In the human being hypophyseal insufficiency is very frequently accompanied by anemia which is ordinarily normochromic There is also a tendency to leucopenia with neutropenia and in occasions eosinophylia The most useful medication seems to be testosterone and cortisone

In hypophysectomized beings anemia is not due exclusively to hypofunction of the thyroid or the testicles since it is more intense than any anemia following castration in the male or thyroidectomy and can be observed equally well in the female

This would make one think of a direct action of the hypophysis on the bone marrow by means of the growth hormone which is capable of increasing the reticulocyte count in males after hypophysectomy or castration

This anemia has been interpreted by some as due to an excessive destruction of the erythrocytes and by others to a diminished production of them but this latter theory is more accepted because of the striking hypoplasia of the bone marrow as well as it s inferior reaction towards hypoxia together with the reticulopenia The hypophysis stimulates erythropoiesis by it s stimulating action on cell growth and protein anabolism

Repeated injections of the posterior lobe extract produce intense anemia in rabbits seemingly due to the consequent oliguria hemodilution and hemolysis Given in smaller doses it may provoke a temporary polycythemia This is also observed after pathologic or experimental lesions of the diencephalon

In rabbits rats and puppies hypophysectomy causes a decrease in weight of the spleen with an increase of the white pulp When given alkaline extract of hypophysis as well as an extract rich in growth hormones the result is a hypertrophy of the spleen In acromegaly the enlargement of the spleen is very frequent

The pars distalis of the hypophysis is one of the physiological factors acting as a continuous regulator on the lymphoid organs as well as on the hemopoiesis and homeostasis of the white blood cells Through it s action on the endocrine glands that produce steroid hormones such as the adrenals testicles and ovaries it works like a brake or moderator in the development of lymphoid tissue stimulating it with the aid of other hormones such as somatotrophin and thyrotrophin The influence of all these factors is strong above all on the thymus and then on the diffuse lymphoid tissue it is less intense on the ganglions and weaker still on the spleen

In the blood the injection of adreno cortico trophin as well as the alarm reaction produce both initial and secondary phenomena The first are of short duration and consist of an increase in neutrophils lymphocytes and eosinophils The second are more characteristic and lasting consisting of lymphopenia and eosinopenia

In human subjects eosinopenia is far more intense characteristic and constant than lymphopenia That is the reason why eosinopenia is preferably used as a test for the discharge of cortico adrenal hormones This eosinopenia produced by adrenal response also depends on peripheral mechanisms which may cause eosinophils to diminish Anyway even though it is not an absolute index of the functional status of the adrenal it is a valuable diagnostic test

The injection of adrenalin is not a specific test to explore adrenal function

13

Endocrine Regulations of Hematopoiesis

WILLIAM DAMESHEK*

THAT definite relationships exist between the various endocrine glands and the blood forming organs is becoming increasingly apparent The hormones ACTH and cortisone apparently stimulate bone marrow activity excessive amounts cause erythrocytosis polymorphonuclear leukocytosis and thrombocytosis although having simultaneously a destructive or inhibitory effect on lymphoid splenic and thymic tissue

A reciprocal relationship between bone marrow and splenic lymphoid tissues and a regulatory or inhibitory effect of the spleen on the bone marrow both seem

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likely One can hypothecate an adrenal cortex–bone marrow–splenic axis " The bone marrow is also influenced somewhat by the thyroid and the gonads the thyroid gland and male sex gland being myelostimulatory whereas the female sex hormone is probably depressant

I *The Normal Range of Hemoglobin and R B C Levels* There is a sex difference in hemoglobin R B C values

	Male	*Female*
Hemoglobin	14 5–16 0	12 5–14 0
R B C	4 6– 6 0	3 3– 4 5

Male castrates have low red counts unusually dynamic males (often with hypertension peptic ulcer, or coronary thrombosis) have relatively high red cell levels Unusually feminine women with very soft skins (stilbestrol excess?) have the lowest red cell counts

II *The Alarm Reaction and Its Effect on the Blood Cells* Various forms of stress and numerous infections (pyogenic) cause a characteristic blood cell picture

Polymorphs +
Lymphocytes –
Eosinophiles 0

In other words polymorphonuclear leukocytosis lymphocytopenia and disappearance of eosinophiles The latter phenomenon is used as a test for ACTH activity

III *The Prolonged ACTH Cortisone action* One of the features of the Cushing syndrome is pancytosis i e erythrocytosis polymorphonuclear leukocytosis and thrombocytosis probably the result of long continued bone marrow stimulation by adrenal cortical hormones (neoplasms prolonged ACTH or cortisone administration)

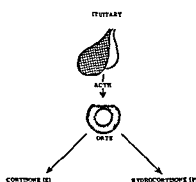


FIG 1 —Anterior Pituitary and Adrenal Cortex The anterior pituitary produces ACTH which stimulates the adrenal cortex to produce cortisone and hydrocortisone

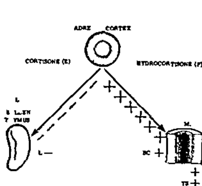


FIG 2 —A reciprocal or antagonistic relationship is present between the lymphoid splenic and thymic tissues and the bone marrow Cortisone depresses the former and stimulates the latter

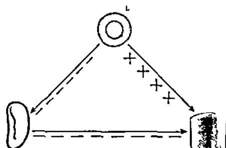


Fig 3 —Adrenal Cortex Spleen—Bone Marrow Axis The adrenal cortex stimulates the bone marrow depresses the spleen but the spleen depresses the bone marrow

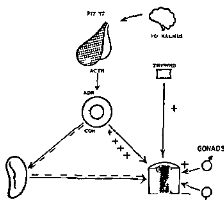


Fig 4 —The Larger Endocrine—Bone Marrow Relationships The bone marrow is related to various endocrine organs some of which stimulate (+) some depress (−)

IV *Prolonged ACTH Cortisone Deficiency* This is seen in chronic hypopituitarism and in Addison's disease Pancytopenia often develops In Addison's disease lymphocytosis and eosinophilia are also present and may be corrected by cortisone

V *Hypersplenism and Hyposplenism* Splenomegaly is usually accompanied with either selective or total cytopenias and a hypercellular marrow (maturation arrest blocked delivery) Splenectomy usually results in a return to normal blood values These observations suggest splenic hormones having regulatory or inhibitory effects on the marrow

Following splenectomy or in splenic atrophy Howell Jolly bodies and lepto

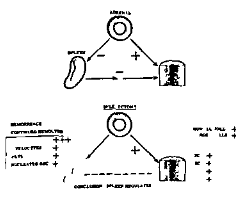


Fig 5 —Spleen and Bone Marrow Following splenectomy bone marrow activity appears to increase With hemorrhage and continued hemolysis after splenectomy bone marrow activity is striking and myeloid metaplasia may occur

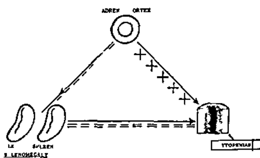


Fig 6 —Hypersplenism Hypersplenism may be conceived of as the action of two or three or more spleens against the bone marrow thus giving an augmentation of the normal effect

FIG 7 —Spleen and Splenic Hormones (Unger) Unger has demonstrated splenic hormones related to the platelets blood vessel wall resistance and fibrinolytic mechanism This work requires confirmation

FIG 8 —Spleen and Splenic Hormones (L O Jacobson) Jacobson has demonstrated splenic hormones with the technic of using X rays and spleen shielding

cytosis develop Splenectomized individuals when stressed (bleeding infection ACTH) show many early red and white cells peripherally indicating a lack of the normal regulatory influence of the spleen

VI *Immuno hematologic and Endocrine Relationships* Cortisone has inhibitory effects on lymphoid splenic thymic and probably plasmocytic tissues which produce certain protein (globulin) fractions some of them antibodies Abnormal antibody production as in auto immune hemolytic anemia is reduced by ACTH with resultant effects on anemia and concentration of free and adsorbed antibody

Immunologic mechanisms are also important in certain cases of thrombocytopenia vascular purpura (Henoch Schonlein) periarteritis nodosa and disseminated lupus The results of ACTH therapy in these conditions are variable although frequently favorable

FIG 9 —The Three Elements in Hypersplenism (1) splenomegaly (2) a full marrow (3) blood cytopenias

FIG 10 —The Cushing Syndrome is characterized by exaggeration of the normal cortisone activity

VII *Endocrine White Cell Proliferative Relationships* The reciprocal relationship between granulocytic and lymphocytic tissues may also hold for granulocytic and lymphocytic leukemia The various myeloproliferative disorders may be due to an unusual myelostimulatory substance

ACTH often reverses the proliferative process in acute childhood lymphocytic leukemia (metabolic relationship?) Combined aminopterin and hormone therapy is particularly valuable Granulocytic leukemia is usually accelerated by ACTH

VIII *Therapeutic Opportunities* Many opportunities for therapy as suggested from the above are available These deal with anemia (hypoplastic hemolytic) splenic neutropenia thrombocytopenia vascular purpura and leukemia

Regulación Endocrina de la Hematopoyesis

Las relaciones existentes entre varias glándulas de secreción interna y los órganos formadores de sangre resultan más evidentes cada día Las hormonas A C T H y cortisona aparentemente estimulan la actividad de la médula ósea cantidades excesivas causan eritrocitosis leucocitosis polimorfonuclear y trombocitosis al mismo tiempo tiene un efecto destructor o inhibidor de los tejidos linfoideos esplénico y tímico Parece existir una relación recíproca entre la médula ósea y los tejidos linfoideos y esplénicos y efectos reguladores o inhibidores del bazo sobre la médula osea Se puede postular un eje cortico adrenal médula osea y esplénico La médula ósea es también influenciada por la tiroides y las gónadas las tiroides y las gonadas masculinas son mieloestimulantes y las gónadas femeninas son quizás depresoras

I *Concentración normal de hemoglobina y nivel de globulos rojos* Hay diferencias sexuales en la hemoglobina y en el numero de glóbulos rojos

	H h	H mb
Hemoglobina	14 5 16 0	12 5-14 0
Glób Rojos	4 6– 6 0	3 3– 4 5

Los machos castrados tienen menos globulos rojos por excepción hombres dinámicos (a menudo con hipertension arterial ulcera péptica o trombosis coronaria) tienen niveles relativamente altos de glóbulos rojos Mujeres excepcionalmente femeninas con piel muy suave (exceso de estilbestrol?) tienen niveles bajos de globulos rojos

II *La reaccion de alarma y sus efectos sobre las celulas sanguineas* Varias formas de stress (estímulos intensos) y numerosas infecciones (piogénicas) provocan un típico cuadro sanguíneo

Polimorfonucleares +
Linfocitos —
Eosinófilos 0

En otras palabras leucocitosis polimorfonuclear linfocitopenia y desaparición de los eosinófilos Este ultimo fenómeno es utilizado como prueba de actividad del A C T H

III *Acción prolongada del A C T H y la Cortisona* Uno de los cuadros del síndrome de Cushing es la pancitosis ej eritrocitosis leucocitosis polimorfonuclear y trombocitosis probablemente el resultado de una larga y continuada estimulación de la médula ósea por las hormonas corticales (neoplasmas administración prolongada de la A C T H y cortisona)

IV *Deficiencia prolongada de A C T H y Cortisona* Esto se ve en el hipopituitarismo cronico y en la enfermedad de Addison A menudo se desarrolla pancitopenia En la enfermedad de Addison se encuentra también linfocitosis y eosinofilia que se corrigen con la cortisona

V *Hiperesplenismo e Hipoesplenismo* La esplenomegalia es acompañada por citopenias electivas o totales e hiperplasia de la médula ósea (detención de la maduracion y bloqueo)

La esplenectomía generalmente corrige y normaliza los valores sanguíneos. La observacion sugiere la existencia de hormonas que tienen efectos inhibitorios o reguladores sobre la médula ósea. Después de la esplenectomía u atrofia esplénica aparecen cuerpos de Howell Jolly y leptocitosis. Individuos esplenectomizados con estímulos intensos (hemorragia, infecciones, ACTH) muestran algunas formas inmaduras rojas y blancas en la sangre periférica indicando una falla de la regulación normal del bazo.

VI. *Relaciones endocrinas e inmunohematologicas.* La cortisona tiene un efecto inhibidor sobre los tejidos linfoideo, esplénico, tímico y probablemente plasmocítico los cuales producen ciertas fracciones proteicas (globulinas) algunas de ellas anticuerpos.

La producción anormal de anticuerpos como en la anemia hemolítica por autoinmunización es reducida por el ACTH con efectos sobre la anemia y los anticuerpos libres o absorbidos.

El mecanismo inmunológico es también importante en ciertos casos de trombopenia y purpura vascular (Henoch Schonlein) periarteritis nudosa y lupus eritematoso generalizado. El resultado con la terapia de ACTH en estas condiciones es muy variable y frecuentemente favorable.

VII. *Relaciones endocrinas de las proliferaciones de las células blancas.* Las relaciones reciprocas entre los tejidos granulocíticos y linfocíticos pueden también ser mantenidas para las leucemias linfáticas y granulocíticas. Los varios procesos mieloproliferativos pueden ser debidos a un desusado estímulo mieloideo. El ACTH a menudo hace reversible los procesos proliferativos en la leucemia linfática aguda de la infancia (relaciones metabólicas?) La combinación de aminopterin con la hormonoterapia es particularmente valiosa. La leucemia granulocítica es a menudo acelerada por el ACTH.

VIII. *Oportunidades terapéuticas.* Muchas oportunidades terapéuticas que sugiere lo anteriormente relatado son consideradas tales como anemia (hipoplástica, hemolítica) neutropenia esplénica, trombocitopenia, purpura vascular y leucemia.

I-4

Nervous Regulation of Hematopoiesis

SVEN MOESCHLIN*

DUE TO the great advances in the field of hormone research during the last few years, rather unjustly the findings reported up to the present in the literature about the nervous regulation of hematopoiesis have remained very much in the background. After the excellent surveys by Castex, Houssay and Dameshek of the influences exerted by hormones we ought to review here the available knowledge about the nervous regulation of hematopoiesis. We must understand clearly however that from the physiological and clinical standpoint the nervous and hormonal regulations are not two separated regulation mechanisms for blood formation, but that both are united in a mutually active fine interplay.

Investigation began in this field with Rosenow's[1] basic establishment that with the so called cerebral puncture in rabbits, i.e. with irritation of the diencephalon (in the regions of the corpus striatum, thalamus and hypothalamus)

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but not with irritation of other parts of the brain a pronounced leukocytosis with shift to the left can be produced Borchardt[3] observed the same phenomenon in cats on puncture of the tuber cinereum The appearance of numerous banded forms indicated that it involved actually a reaction of the bone marrow and was not due to alteration merely in distribution of leukocytes (fig 1) The leukocytoses thus produced clearly exceed spontaneous fluctuations and appear regularly shortly after the stimulus reaching their highpoints approximately one hour later The leukocytosis as *Rosenow*[1] emphasized was not always accompanied by a hyperpyrexia and therefore these centrally released leukocytoses could not be interpreted as the result of hyperpyrexia Rosenow[4] could show also with certainty that not every hyperpyrexia is accompanied by a leukocytosis Thus the injection parenterally of beta tetrahydronaphthylamin which produces an intense increase in body temperature in rabbits has no influence on the number or distribution of the leukocytes (Rosenow[4]) The intraventricular injection of kaolin into the cerebrospinal fluid of rabbits was able to release somewhat slower the same leukocytosis (Rosenow[6]) It remains obscure whether this involves also a specific irritation of a definite part of the brain or is only a reaction to a foreign body

This finding of a centrally produced leukocytosis released by stimulation of the midbrain has been confirmed by numerous other workers Urra and co workers[7] Riccitelli[8] Shinosaki[9] and co workers Sakurai[10] Aburaya[11] and Ha

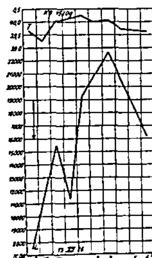


Fig 1 —Induced neutrophilic leukocytosis by puncture of the r thalamus (rabbit) The peak of the leukocytosis is reached in 6 hours (from G Rosenow Zeitschr f d ges exp Med 64 456 1929 Verlag Julius Springer)

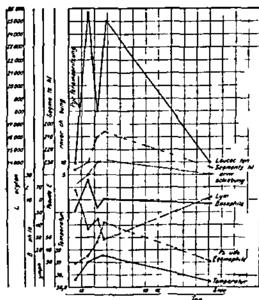


Fig 2 —Leukocytes response on the intravenous Pyrifer injection of the rabbit (from Hoff)

yashida,[12] Hoff,[13] Ginzberg and Heilmeyer[14] observed also a distinct leukocytosis in the peripheral blood following air filling of the cerebral ventricles. Wespi[15, 16] worked with the technique developed by the Swiss Nobel prize winner Hess[17] of stimulating or destroying by means of a fine diathermy needle spots which could be identified later histologically in the brain of cats. He showed that the points releasing a leukocytosis were actually in the midbrain and with that proved conclusively that the midbrain plays an important rôle in the regulation and production of leukocytes. It would be false from the existing evidence to speak of an actual leukocyte regulation center in the midbrain. To do so would be to contradict the basic experimental results of Hess[13] regarding vegetative regulation, since an interplay of various coordination centers seems far more probable for so complicated a system.

For the further investigation of these regulatory mechanism we are especially indebted to the experiments of Hoff[13, 19, 20] and his co-workers. He showed that the injection of killed bacteria or their products could produce experimentally a pronounced leukocytosis. In very thorough investigations he was able to show for the first time that there appeared a quite regular course of events in the qualitative and quantitative aspects of the blood picture. There occurs for example in rabbits following the intravenous injection of Pyrifer (fig. 2) (a killed vaccine of nonpathogenic coli bacilli) first a pronounced granulocytopenia with a disappearance of the eosinophiles and also a lymphopenia. This initial depressive action corresponds in his opinion to a sympathetic stimulation and is followed after 1–2 hours by the actual irritation phase through increased excitation of the vagus. That is, the granulocytes now increase sharply with the banded forms especially showing an increase. After several hours this reaction also dies away and there appears an increase in the monocytes and later too

1st Phase	2nd Phase
Rise of fever, height of fever	Decrease of fever
Increase of leukocytes	Decrease of leukocytes
Increase of granulocytes	Increase of lymphocytes
Decrease of eosinophiles	Increase of eosinophiles
Increase of reticulocytes	Decrease of reticulocytes
Decrease of alkali reserve (acidosis)	Increase of alkali reserve
Increase of metabolic rate	Decrease of metabolic rate
Increase of serum proteine	Decrease of serum proteine
Decrease of albumine globulin ratio	Increase of albumine globulin ratio
Increase of blood sugar	Decrease of blood sugar
Decrease of blood fat	Increase of blood fat
Decrease of blood cholesterol	Increase of blood cholesterol
Increase of ketone bodies	Decrease of ketone bodies
Increase of blood creatine	Decrease of blood creatine
Change of K/Ca ratio	Change of K/Ca quotient
Predominance of sympathetic system	Predominance of parasympathetic system
ERGOTROPHIC PHASE (Hess)	TROPHOTROPIC PHASE (Hess)

FIG. 3.—Scheme of Vegetative Regulation (from Hoff)

the reappearance of eosinophiles and finally an increase in lymphocytes which Naegeli [1] had already mentioned as postinfection lymphocytoses If one employs for both phases the idea featured by W R Hess [18] one can speak then of an initial *ergotrope* and a later *trophotrope* phase (fig 3)

We ourselves (Moeschlin[27]) have studied the effect of the injection of Pyrifer in humans and have developed it as a clinical functional test of the bone marrow The injection of 25 units of Pyrifer i v in the morning and the checking of the leukocyte numbers every 2 hours during the following next 10 hours give normally the curve seen in fig 4 left In a damaged bone marrow on the other hand (for example benzene intoxication etc) the test gives a very flat curve as seen in fig 4 right

This regularity of the leukocyte curve agrees well with that previously mentioned by Schilling [3] for the blood picture of man in acute infection Both observations are identical with the currently presented blood changes under the *influence of stress* described by Selye [4] *as adaptation syndrome* and widely known today To recent investigators doubtlessly belongs the merit of studying closely the course of the different phases and bringing them for the first time in connection with the hypophysis and adrenals

Let us return now however to the further investigations of Hoff and Rosenow In the experimental study of the leukocytosis released by Pyrifer Rosenow (fig 5) noticed that this experimental infection leukocytosis could be stopped by administration of larger doses of brain stem narcotics such as Luminal Paschkis [6] was able to confirm these findings On the contrary Rosenow[25] found

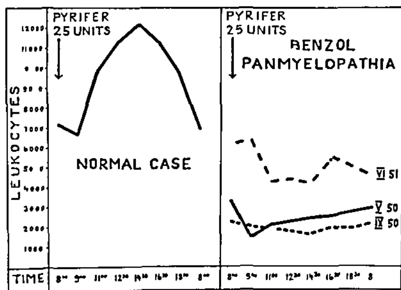
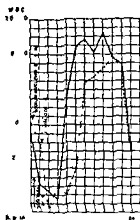
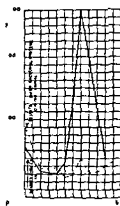


Fig 4—Functional bone marrow test with intravenous injection of 25 units of Pyrifer *Left* The normal curve *Right* Typical very flat curve in the case of benzol intoxication in different intervals The curve is slightly improving after one year of observation (see Moeschlin Helv Med Acta *1* 299 1945)

that although antipyrin stopped the rise in temperature it did not hinder the appearance of leukocytosis and it can be derived from this that also with Pyrifer the leukocytosis is not produced by the temperature increase In contrast to the effect of these midbrain narcotics other preparations that affect the cerebrum (chloreton and chloral hydrate) have no influence (Fig 6) It therefore follows that stimulation from the midbrain may be postulated also for the regulatory mechanism of the leukocytosis released by infection The question now must be further clarified in what manner this stimulation from the midbrain succeeds in the production of the characteristic blood picture

Hoff[19, 20] and Hasabida[1] showed that following section of the cervical spinal cord the Pyrifer or brain puncture leukocytosis was absent or appeared only weakly However if the cervical spinal cord was only cut the half way on one side the leukocytosis was still present (fig 7) Maeda[27] showed the same for the kollargol leukocytosis Muto found[8, 9] that the section of the cervical spinal cord also hindered the leukocytosis following typhus vaccine sodium nucleinate arsenite electralgol and burning of the skin Certainly it follows that the leukocytosis is not explained by the stimulation released from the midbrain of the hypophysis and the thus produced ACTH secretion Rather it is certain that stimulations are transmitted to the blood building organs by nervous pathways Pasztor Izsak and Martin[10] assume that the nervous stimulation travels over the sympathetic ganglion chain since following bilateral sympathectomy the Pyrifer leukocytosis is absent





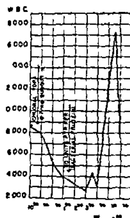


FIG 5 —*Left* Dog 9 5 kg Leukocytosis induced with sod nucleinate (solid line) Leukocytosis with sod nucleinate 5 days later while the dog was under luminal narcosis (broken line) *Center* Rabbit 86 2700 g Leukocytosis by bacterial protein (solid line) Rabbit 104 2500 g Curve of leukocytes after inj of the same amount of bacterial protein while the animal was under luminal narcosis (broken lin)

FIG 6 —Rabbit 91 2200 g Narcosis by chloral hydrate induces a leukopenia Bacterial protein injection is later followed by a very marked neutrophilic leukocytosis

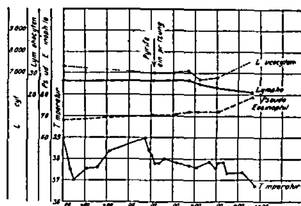


Fig 7 —Pyrifer leukocytosis is absent if the cervical spinal cord is sectioned (Hoff)

Numerous workers have investigated the effect of vagal or sympathetic stimulation on the blood picture The results, however, were quite different according to the animals used or the technique of the experiment which well may be due to the fact that the effect on the hormone producing organs was likewise very different in the various animals and under varying conditions of the experiments These experiments therefore cannot be critically compared though Hortling[31] has reviewed them closely

The question remains open whether central nervous system stimuli go directly over the sympathetic chains to the bone marrow as Hayashida[1] supposes or whether also here again a further relay station is inserted between the sympathetics and the bone marrow

Beer[31, 32, 33] a student of Hoff was able to show that a hormonal factor is required for the release of leukocytosis by splanchnic stimulation He placed

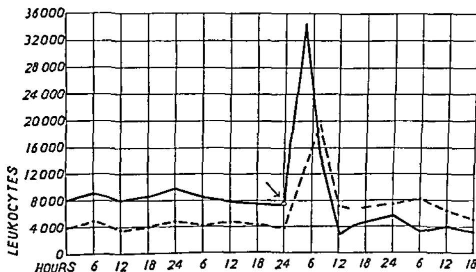


Fig 8 —Two rabbits in parabiosis Induced leukocytosis in one partner is promptly followed by a leukocytosis of the same order in the parabiont (from Beer)

two animals in parabiosis When he produced a leukocytosis in one animal by ventricular puncture or by intravenous injection of bacterial residue there appeared somewhat later a marked leukocytosis in the parabiont (fig 8) Since the two animals did not have a common circulatory system and the leukocytosis followed ventricular puncture it is postulated that a diffusible leukocyte producing hormone called forth in the stimulated animal is concerned Beer[31, 32, 33] also could transmit this substance with the plasma of one animal to another and he called this leukocyte producing factor *leukopoietine*

How does the secretion of this leukopoietine take place? In systematic experimental investigations he finally was able to prove that the nervous stimulation from the midbrain is carried first over the sympathetics to the splanchnic nerves and from here reaches the liver Here it stimulates the secretion of leukopoietine which affects then by way of the circulating blood a stimulation of the bone marrow i e an increased production and release of granulocytes *If one removes the abdominal organs in series one after another the experimentally induced leuko cytosis is preserved however after denervation of the liver it appears only in very slight degree* and generally no longer is found in the parabiont at all The e results of splanchnic section were fully confirmed by the experiments of Muto[8, 9] and Saheki[35] Saheki could show in addition that in experimental damage of the liver by phosphorus without demonstrable damage to other organs the test leukocytosis with nucleinates was negative

Besides the transmission of the leukocyte stimulating effect through the sympathetic fibers over the splanchnic nerves to the liver and the secretion of leukopoietines we however also must suppose a direct transmission through nervous fibers to the bone marrow Thus it was shown for example in the experiments of Beer[31, 32, 33] that even after section of the splanchnic nerves a slight leukocytosis appears following injection of Pyrifer (the increase reached then only 50% in contrast to 200–300% with intact splanchnics!) It is possible that such directly transmitted nervous stimulation of the bone marrow is carried over the parasympathetics and leaves over the posterior roots of the spinal cord to go directly to the bone marrow Thus Morikawa[37] observed in stimulation of the parasympathetics from L 5 to L 7 and S 1 a marked increase of metamyelocytes and banded neutrophiles in the blood from the femoral vein of the stimulated side This however was lacking in blood from the femoral vein of the non stimulated side In several cases there appeared also an increase of thrombocytes and reticulocytes Okinaka and co workers[38] found in addition that severing all sympathetic fibers leading to the bone marrow produced an increased cellularity and a decrease in fat cells of that marrow The section of the spinal parasympathetics lead on the other hand to a decrease of the active bone marrow and to an increase of the inactive fat marrow Probably similar behavior holds true for the lymphocytes Florey[39] found by irritation of a nerve to a lymph node that an increase in lymphocytes appeared in the vein draining the node (previously 14–20 000 afterwards 88 000)

The conclusion can probably be drawn from the various investigations that the bone marrow on the one hand can be directly stimulated in slight degree



Fig 7 —Pyrifer leukocytosis is absent if the cervical spinal cord is sectioned (Hoff)

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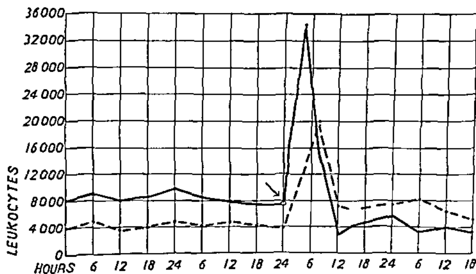


Fig 8 —Two rabbits in parabiosis Induced leukocytosis in one partner is promptly followed by a leukocytosis of the same order in the parabiont (from Beer)

Rosenow [5] in experiments not yet published in detail was able by injecting cerebrospinal fluid from a rabbit or a dog with a neutrophile leukocytosis to produce in the recipient animal a blood leukocytosis following a latent period As long however as these results and especially simultaneous controls with normal cerebrospinal fluid are not published we can not take a stand on this The previous experiments presented in the literature speak rather against such a regulation by the cerebrospinal fluid

Regulation of the Remainder of the Blood Cells Erythropoiesis and thrombocytopoiesis are probably increased by a similar nervous regulatory mechanism Probably the midbrain plays an important role here too Thus Da Rin and Costa[44] observed pronounced reticulocytosis with midbrain puncture technique Ginzberg and Heilmeyer[14] with encephaloventriculography as well as lumbar and occipital puncture observed the same Dockhorn[45] likewise found an increase of reticulocytes with stimulation of the midbrain region with diathermy

Correlation of the Above Mentioned Nervous Mechanism of Regulation with the Hormones Secreted by the Hypophysis and the Adrenal Glands From the recent investigations of Selye [4] and other investigators there remains no doubt that after injection of bacterial material (Pfeffer and Staudinger [46] Keiderling and Westphal[1]) an increased excretion of 11 oxycorticoids is found in the urine There occurs for example with Pyrifer injection an increased secretion of ACTH and thereby an increased output of cortisone into the blood Does stimulation of the diencephalic region lead to leukocytosis by stimulation of the hypophysis and ACTH excretion? The question is how far this stimulation of the anterior lobe of the hypophysis is coupled with or independent of the above mentioned nervous regulation of the blood

According to Selye [4] the actual adaptation syndrome is absent following extirpation of the hypophysis or adrenal glands This does not hold for the appearance of the irritation leukocytosis as Hoff[0] recently emphasizes Thus Beer and Bedacht[48] in 1941 were able to show that in adrenalectomized rabbits after a central nervous stimulation a pronounced leukocytosis still appears if one waits until the animal recovers from the shock attendant on the operation on the adrenal glands This has recently been confirmed also in adrenalectomized rats by the investigations of Lewis and Page[49] with Pyrifer and by Langendorff and Tonutti[50] with cold stimulation in which a decided leukocytosis was observed Langendorff and Tonutti[50] no longer observed in this case a lymphopenic reaction while Lewis and Page[49] in rats could still demonstrate the lymphopenia

The same applies for the hypophysectomized animal Already in 1945 Velasco and Romo[51] showed that the leukocytosis following ventriculography can still be released in hypophysectomized dogs Schmert[5] found that the Pyrifer leukocytosis in hypophysectomized rabbits returned after one week to 30–70% contrasted with 200% before operation After four weeks this reaction disappears completely and can be produced again only with a pre treatment of the animal with ACTH *ACTH or some other substances present in these extracts seems therefore in some way necessary for the normal function of the bone marrow although it does not play a decisive role in the release of the irritation leukocytosis* That mecha

over the parasympathetic fibers and inhibited by the sympathetic fibers entering the bone marrow with blood vessels On the other hand a strong reaction of the bone marrow occurs only when the humoral factor released from the liver is added These mechanisms have been summarized in fig 9

Thus we have here also in the final analysis as generally in neurophysiology the combination of nervous and humoral factors in transmission of the stimulus

As yet nothing further is known about the actual *chemical nature of leukopoietine* Menkin[40] found a leukocytosis inciting factor in exudates which is composed of a water soluble polypeptide Abderhalden[41] and Elsasser[4] isolated a leukocyte stimulating material from urine Prf Turner and Miller[43] also found such a substance in extracts of urine It is possible that one of these substances is identical with the *leukopoietine* secreted by the liver however we are not certain of that yet and one has to be very critical on this point because of the non specific leukocyte stimulating effect of many chemical compounds injected intravenously

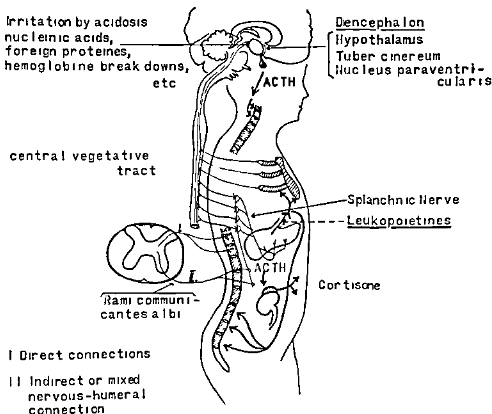


FIG 9 —The nervous regulation of the hematopoiesis From the diencephalic centers nervous connections go through the spinal cord (*I*) Direct connections are carried over posterior roots (parasympathetic fibers) to the bone marrow with stimulating effect Depressive effects probably go over sympathetic fibers to the bone marrow (*II*) An indirect or mixed nervous humoral connection exists between the anterior roots and the sympathetic ganglions over the splanchnic nerve to the liver and giving rise to an out put of leukopoietins in the blood with stimulating effect on the bone marrow

of thrombocytes and erythrocytes The nervous regulatory mechanisms described here appear also in hypophysectomized animals when the initial shock reaction has been overcome and the hypophyseal hormones have not been deficient too long They are consequently not directly dependent upon the ACTH production of the hypophysis and represent independent mechanisms of regulation

La Regulación Nerviosa de la Hematopoyesis

Durante los ultimos veinte anos investigaciones detalladas acerca del mecanismo de regulación de los leucocitos en el transcurso de las infecciones han sido hechas sobre todo por Hoff y sus discípulos

Habiendo enseñado Rosenow que después de pinchar el cerebro en un lugar determinado se observa el fenómeno de una leucocitosis considerable unida eventualmente a una elevación de temperatura pareció posible que el hipotálamo tuviera gran importancia con respecto a la regulación de los leucocitos Wespi pudo afirmar usando la técnica desarrollada por Hess de irritación o destrucción de lugares determinados del cerebro con electrodos finísimos que irritando lugares precisos del hipotálamo se puede comprobar una leucocitosis muy destacada Quiso deducir de ello la existencia de un centro de regulación para los leucocitos situada en el hipotálamo lo que sin embargo se opone a la tesis de Hess que sostiene que tratándose de un mecanismo de regulación complicado supone más bien una cooperación entre varios centros coordinados Hoff pudo indicar que una leucocitosis muy importante puede ser producida por inyección de bacterias o de sus productos de desintegración y por primera vez logró enseñar en investigaciones muy detalladas y lógicas que después de dichas inyecciones la fórmula sanguínea en lo que se refiere a los leucocitos tomaba un transcurso determinado con respecto a cantidad y calidad de estas células Inmediatamente después de una inyección de Pyrifer (una vacuna de bacterias coli) se produce una granulocitopenia destacada una linfopénia y desaparición de las células eosinófilas Una a dos horas más tarde se presenta una granulocitosis acentuada es decir una elevación del número de neutrófilos sobre todo de formas jóvenes con nucleos abastonados Al transcurrir algunas horas esta reaccion desaparece y se produce una elevacion eventual del numero de monocitos luego reaparición de células eosinófilas y por fin una linfocitosis postinfecciosa Esta regularidad coincide ampliamente con los cambios sanguíneos humanos determinados por Schilling durante enfermedades infecciosas agudas y ambas observaciones coinciden tambien con otras modernas conocidas hoy dia como la reacción de stress de Selye Hoff y sus discípulos pudieron enseñar a su vez que esta leucocitosis debida a una infección experimental no se establece en cuanto se corta la médula oblonga (Hava hida) o se extirpa bilateralmente el sistema nervioso simpático (Pasztor Issak y Martin) Por consiguiente ciertos impulsos nerviosos deben ser dirigidos a los órganos hematopoyéticos impulsos que en parte son responsables de que los leucocitos sean arrastrados fuera de la médula ósea

Como Rosenow y sus colaboradores demuestran que por narcosis del hipotálamo también se puede bloquear una leucocitosis experimental mientras que la narcosis cortical no tiene efecto Hoff logro demostrar en trabajos ulteriores con Beer que el impulso nervioso es dirigido por los nervios del esplácnico hasta el hígado el cual a su vez libera una sustancia llamada *leucopoietina* capaz de estimular la médula osea y causar una producción aumentada y liberación de granulocitos en la sangre circulante Si se elimina un organo abdominal tras otro a excepcion del higado la leucocitosis experimental queda conservada Beer pudo demostrar que inyectando sustancias provenientes de bacterias a uno de dos animales unidos entre si en forma de parabiosis (que por lo tanto no tienen la misma circulación de la sangre) y causando así una leucocitosis después de un intervalo determinado también se declara una leucocitosis en el segundo animal Esta leucocitosis por irritación era transferida de un animal al otro mediante el plasma lo que demuestra que este fenómeno se debe a una sustancia especial llamada hoy *leucopoietina* Investigaciones experimentales de otros autores (Saheki) confirman estas suposiciones Hasta ahora no se conoce

nism is explained by the above described transmission of stimulation by way of nervous pathways

Recently Hubler, Higgins and Herrick[53] have carried out a systematic investigation of the influence of diathermy hyperthermia in adrenalectomized hypophysectomized hypophysectomized adrenalectomized and adrenalectomized demedullated rats They found that the neutrophile reaction released by increase of body temperature to 41–41 5 C remained after adrenalectomy that however the lymphopenia and eosinopenia appearing in the control animals here disappeared *In the animals that were both adrenalectomized and hypophysectomized* the artificial increase in body temperature was lacking as well as the eosinopenia and lymphopenia It may be assumed probably in analogy to the investigations of Schmert[5] that here also the function of the bone marrow was reduced due to the long absence of hypophyseal action and would have been restored with an artificial addition of ACTH *From the results available at present it seems that the hypophysis is not absolutely necessary for the direct transmission (to the bone marrow) of the leukocyte stimulation that however the activity of the bone marrow probably expires after some time in hypophysectomized animals and can be restored later only with artificial addition of ACTH*

Destruction of the Blood Cells In conclusion it might be mentioned that not only the production but very probably also the destruction of the blood cells is regulated through nervous mechanisms although we do not know anything further about this It is quite remarkable how exactly the normal values of the different blood cells are maintained in man both quantitatively and qualitatively A transient polyerythrocytemia or leukocytosis incited by transient stimulus disappears very quickly more quickly than the average life span of the cells involved It must be supposed that not only the release of these cells can be stimulated or inhibited but that also for the quick return to normal value the life span and the elimination of these cells can be subjected to regulatory influences The spleen probably plays a role in the elimination and destruction of erythrocytes and thrombocytes From recent investigations by Weisberger Heinle and Hannath[54] Weisberger and Heinle[55] Lanman[56] Bierman[57] and coworkers it appears that especially the lung capillaries undertake this task for the granulocytes and to only a small degree are the liver and spleen involved Whether and in what way a nervous regulation is concerned in this is unknown at present

Summary

On the basis of experimental investigations reported in the literature to the present a review of the nervous regulation of the blood cells is given The production and release of blood cells from the bone marrow is controlled on the one hand by regulatory centers in the midbrain that is to say the parasympathetic fibers travelling from the spinal cord directly to the bone marrow can incite blood formation and release the sympathetic fibers entering the bone marrow with blood vessels can inhibit these processes On the other hand with strong stimulation in the region of the midbrain for example by bacterial substance increased blood production and release is effected via sympathetic stimulation over the splanchnic nerves to the liver where *Leukopoietin* is secreted and carried in the circulation to the bone marrow Similar conditions probably hold true for the production and release

of thrombocyte and erythrocytes The nervous regulatory mechanisms described here appear also in hypophysectomized animals when the initial shock reaction has been overcome and the hypophyseal hormones have not been deficient too long They are consequently not directly dependent upon the ACTH production of the hypophysis and represent independent mechanisms of regulation

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la naturaleza de estas sustancias pero es posible que sean idénticas a la sustancia aislada de la orina por Abderhalden y Elsaesser

Probablemente mecanismos parecidos a los descriptos son responsables de la regulación de la eritropoyesis y trombopoyesis

Basándose en las investigaciones de Selye y sus discípulos se debe suponer hoy que además de la liberación de una sustancia estimuladora de la médula ósea como consecuencia de una irritación nerviosa del hipotálamo exista también un efecto hormonal directo sobre ciertas células sanguíneas y lugares de su formación que no serán discutidos aqui

En conclusion se puede decir hoy que para la regulación de las variaciones en la formula sanguínea durante infecciones agudas y cronicas se puede comprobar además de una regulación nerviosa partiendo del hipotálamo también una regulación hormonal debida al lóbulo anterior de la hipofisis a la glándula suprarenal y al hígado

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I communication I

The Regulation of Hemopoiesis by the Hypophysis

P INTROZZI

Experiments have been performed on guinea pigs to study the influence of prolonged administration of extracts of pituitary hormones on peripheral blood and homopoietic organs (marrow spleen and lymph nodes) With any of the treatments performed it was found that the red cells the hemoglobin and the platelets do not suffer any change A marked and prolonged reticulocyte reaction is observed with the galactogenic hormone with the follicule stimulating gonadotropic hormone and in lesser degree with the luteinizing hormone The white cells have a tendency to increase in number This being more marked after the administration of the somatotrope hormone prolan A and any total extract of the anterior part of the hypophysis The variations in the leucocytic differential formula are more important While the total pituitary extracts the anterior and post rior pituitary extract the intermedium pituitary extract and ACTH will produce neutrophilia and lymphocytopenia the growth hormon s the two gonado stimulating hormones and the galactogentic hormone are characterized hematologically by lymphocytosis sometimes very marked with a normal or slightly increased number of neutrophils Together with these variations of the peripheral lymphocytosis some alterations may occur in the lymphatic organs in which the changes may be sometimes of the regressive type with atrophy of the follicules and signs of histiocytic reaction and other times hyperplastic with hyper trophy of the lymphatic tissue Other interesting findings are the eosinopenia of ACTH the eosinophilia of prolactin and prolan A and the monocytosis of prolan A prolan B and prolactin Disregarding the experiments with the ultrafilterable fraction of the ACTH

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la naturaleza de estas sustancias pero es posible que sean idénticas a la sustancia aislada de la orina por Abderhalden y Elsiesser

Probablemente mecanismos parecidos a los descriptos son responsables de la regulación de la eritropoyesis y trombopoyesis

Basándose en las investigaciones de Selye y sus discípulos se debe suponer hoy que además de la liberación de una sustancia estimuladora de la médula ósea como consecuencia de una irritación nerviosa del hipotálamo exista también un efecto hormonal directo sobre ciertas células sanguíneas y lugares de su formación que no serán discutidos aquí

En conclusion se puede decir hoy que para la regulación de las variaciones en la formula sanguínea durante infecciones agudas y cronicas se puede comprobar además de una regulacion nerviosa partiendo del hipotálamo también una regulación hormonal debida al lóbulo anterior de la hipofisis a la glándula suprarenal y al hígado

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I communication 2

Hematologic Response to Hemolytic Agents in Intact and Adrenalectomized Rats

M RACHMILEWITZ J D FELDMAN O STEIN Y STEIN and A DE VRIES*

Hemolysis was induced by phenylhydrazine in sham operated and adrenalectomized rats It was found

1 The anemia was significantly greater in the adrenalectomized rats

2 The total leucocyte rise was greater in the adrenalectomized rats

3 A transitory drop in eosinophils was observed in the sham operated rats followed by a striking eosinophilia

4 In the adrenalectomized rats there was an immediate eosinophilia which gradually rose to very high levels

5 It was demonstrated that the eosinophilia was not due to hypofunction of the adrenal cortex

6 Histological and biochemical examinations of the adrenal cortex failed to show evidence of hyperfunction of the adrenal cortex during the period of the hemolytic anemia

The hematologic response to other hemolytic agents in adrenalectomized rats is being investigated

Respuesta Hematológica a los Agentes Hemolíticos en Ratas Normales y Adrenalectomizadas

Se provocó hemólisis con fenilhidrazina en ratas adrenalectomizadas y en testigos con operación simulada

Se encontró

1 Que la anemia era significativamente mayor en las ratas adrenalectomizadas

2 El aumento total de leucocitos era mayor en las ratas adrenalectomizadas

3 Una caída transitoria en los eosinófilos fué observada en las ratas con operaciones simuladas seguida de una fuerte eosinofilia

4 En las ratas adrenalectomizadas había eosinofilia inmediata la que gradualmente aumentó a niveles muy altos

5 Se demostró que la eosinofilia no es debida a la hipofuncion de la corteza adrenal

6 Los exámenes histologicos y bioquímicos de la corteza adrenal no mostraron evidencia de hiperfuncion corticosuprarrenal durante el período de la anemia hemolítica

La respuesta hematológica a otros agentes hemolíticos en ratas adrenalectomizadas está siendo investigada

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which will give a hyperplastic reaction of the reticuloendothelial system the other methods of treatment did not cause any changes in the marrow either morphological or cyto functional capable of influencing the peripheral blood picture Rather than having an influence on the hemopoietic activity of the marrow pituitary extract has a definite influence on the lymphocytopoiesis which will be inhibited by the use of ACTH total anterior posterior and intermedium pituitary extract or stimulated such as happens with the administration of somatotropic hormone prolactine and gonadotropine

HIPÓFISIS Y HEMATOPOYESIS

Ha sido estudiado experimentalmente en la cavia la influencia de la administración prolongada de extractos de hormonas hipofisarias sobre la crasis sanguínea periférica y sobre los órganos hematopoyéticos (médula bazo y ganglios linfáticos) Cualquiera que sea el tipo de tratamiento practicado los glóbulos rojos la hemoglobina y las plaquetas no sufren habitualmente modificaciones dignas de mención Una vivaz y prolongada reacción reticulocitaria se observa con la hormona galactógena con la gonadotrofina folículo estimulante y en grado menor con la hormona luteinizante

Los glóbulos blancos globalmente considerados tienden en general al aumento los incrementos más marcados se registran después de suministrar hormona somatotropa de prolan A y de cualquier extracto anterohipofisario total

Mayor importancia revisten todavía las variaciones del cuadro leucocitario diferencial mientras los extractos de hipofisis total de lóbulo anterior y posterior también la intermedina y el ACTH provocan neutrofilia y linfopenia la hormona del crecimiento las dos gónadoestimulinas y la hormona galactogena están caracterizadas hematologicamente por linfocitosis a menudo de grado notable con una tasa de neutrófilos ya invariable o ligeramente aumentada A las variaciones de la linfocitosis periférica se agregan alteraciones de los órganos linfáticos en los cuales es posible observar ora alteraciones regresivas con atrofia de los folículos y signos de reacción histioide ora en vez fenómenos de hiperplasia e hipertrofia del tejido linfoide

Otros hallazgos que merezcan ser señalados en el ámbito de las variaciones leucocitarias son la eosinopenia del ACTH la eosinofilia de la prolactina y del prolan A y la monocitosis del prolan A prolan B y prolactina

Si se prescinde de los experimentos con la fraccion ultrafiltrable del ACTH con la cual se tiene una reacción hiperplástica de los elementos del retículo endotelio a menudo de notables proporciones con ninguno de los otros tratamientos se observan a expensas de la modificaciones morfológicas o citofuncionales tales de incidir en manera apreciable sobre el patrimonio hematológico periférico Más que sobre la actividad hematopoyética de la médula osea el principio hipofisario ejerce una influencia evidente sobre la linfopoyesis la cual es ora inhibida como después del tratamiento con ACTH extractos hipofisarios totales anteriores posteriores intermedios ora exaltada como después de suministrar hormona somatropa prolactina o gonadotropina

Communication 4

Hemostasis in Sympathectomized and Adrenalectomized Animals

ALFRED L. COPLEY and PAUL L. STEFKO*

1 Three hundred and seventy six bleeding times were made in 76 rats after adrenalectomy, thoraco lumbar sympathectomy or both. Only 3 values were markedly prolonged.

2 Twenty one bleeding times in 5 sympathectomized and 4 adrenalectomized rabbits were all within the normal range of 3 minutes.

3 Eight adrenalectomized and 9 sympathectomized rats and 4 adrenalectomized rats previously sympathectomized were all irradiated with 700 r. Bleeding times were within 3 minutes on each of the first 4 to 5 days after irradiation. One out of 4 animals tested on the seventh postradiation day had a markedly prolonged bleeding time.

4 A high incidence of decreased clot resistance was observed in both sympathectomized and adrenalectomized animals.

5 Direct microscopic observations in the capillary beds of the adrenalectomized or sympathectomized hamsters cheek pouches and of the mesentery of sympathectomized rats were made utilizing a double diaphragm method of oblique illumination hitherto not applied to biological materials.

6 The relationship of platelet agglutination thrombi and vasoconstriction in hemostasis is discussed. A new explanation for the narrowing of capillary blood vessels following injury is proposed, i.e. the contraction of an adherent platelet agglutination thrombus.

Hemostasia en Animales Simpatectomizados y Suprarrenalectomizados

1 Trescientos setenta y seis tiempos de sangría fueron practicados en 76 ratas después de la suprarrenalectomía, simpatectomía o ambas intervenciones. Tan solo tres valores fueron marcadamente prolongados.

2 Veintiun tiempos de sangría en cinco conejos simpatectomizados y cuatro suprarrenalectomizados mostraron tiempos dentro de los limites normales de 3 minutos.

3 Ocho ratas suprarrenalectomizadas y nueve simpatectomizadas y cuatro ratas suprarrenalectomizadas con simpatectomía previa fueron irradiadas con 700 r.—Los tiempos de sangría se encontraban dentro de los 3 minutos en los cuatro o cinco primeros días despues de la irradiación. Uno de los cuatro animales examinados al séptimo día de la irradiación tenía un tiempo de sangría marcadamente prolongado.—

4 Tanto en los animales suprarrenalectomizados como en los simpatectomizados fue observada con gran frecuencia disminución de la resistencia del coágulo. —

5 Se realizaron observaciones microscópicas directas en los lechos capilares de las bolsas de las quijadas de los "hamsters" suprarrenalectomizados o simpatectomizados y del mesenterio de las ratas simpatectomizadas utilizando un método de doble diafragma de iluminación oblicua hasta ahora no aplicado a los estudios biologicos. —

6 Se discute la relación de los trombos de aglutinación de las plaquetas y la vasoconstricción en la hemostasia. Se propone una nueva explicación del estrechamiento de los capilares después de una lesión: la contraccion de un trombo de plaquetas aglutinadas y adherentes.

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I communication 3

Participation of Glandular Mucoprotein of Human Stomach in Hematopoiesis and Its Dependence upon Central Nervous Stimulation

GEORGE B. JERZY GLASS, LINN J. BOYD and MICHAEL A. RUBINSTEIN*

The failure of adequate hematopoiesis in pernicious anemia in response to moderate oral doses of vitamin B_{12} is correlated with the absence of intrinsic factor and probably deficient intestinal absorption of B_{12}. Recently our group has shown that glandular mucoprotein, one of the mucous components of normal human gastric juice, resembles Castle's intrinsic factor in its physical and physiological properties. This mucoprotein derives from fundal glands which undergo atrophy in pernicious anemia and is consistently absent from gastric juice in this disease. Its secretion is strongly dependent upon central vagal stimulation. Its concentration in the gastric juice is highest during hypoglycemia caused by intravenous administration of insulin, especially in patients with duodenal ulcer.

In two individuals with macrocytic nutritional non-pernicious anemia, whose gastric juice contained glandular mucoprotein, daily administration of 10–20 mcg B_{12} orally alone was followed by a prompt hematopoietic response. In seven patients with pernicious anemia whose gastric juice did not contain mucoprotein, B_{12} in daily doses of 10–30 mcg was ineffective, but when added daily to 50–200 mg mucoprotein processed from normal human gastric juices, an optimal or suboptimal hematopoietic response was observed in all and complete clinical remission in five of these cases.

These findings indicate a close relationship of glandular mucoprotein to Castle's intrinsic factor, the nature of which is yet undetermined.

Participación de Mucoproteína Glandular del Estómago Humano en la Hematopoyesis y sus Dependencias de los Estímulos Nerviosos Centrales

En anteriores estudios Glass y Boyd separaron de un complejo de compuestos de mucus del jugo gástrico una substancia mucosa que denominaron mucoproteína glandular, porque se origina en el cuello de las glándulas fúndicas del estómago humano.

En estudios posteriores demostraron que la mucoproteína glandular tiene muchas propiedades físicas y fisiológicas que recuerdan al factor intrínseco hematopoyético de Castle. Ambos, la mucoproteína glandular y el factor intrínseco no se encuentran en la saliva, bilis y secreción duodenal humanas. Ambas se forman en el fundus y cuerpo del estómago humano, en esta área es donde se observan las lesiones atróficas más severas en la anemia perniciosa. Más aun la mucoproteína glandular como el factor intrínseco están ausentes o sólo existen en pequeñas trazas en el jugo gástrico de los enfermos con anemia perniciosa, aun si para su secreción se aplican intensos estímulos nerviosos centrales como es la administración de insulina endovenosa. Esto contrasta con la presencia constante de la mucoproteína glandular en el jugo gástrico de enfermos que no tienen anemia perniciosa. Estos estudios fueron llevados a cabo en 9 enfermos con anemia perniciosa no tratados o en recaída, que presentaron los signos y síntomas clásicos de esta enfermedad. Dos de los nueve casos no tuvieron una respuesta hematopoyética adecuada. Estos hechos indican que la mucoproteína glandular, fracción de la mucina gástrica disuelta, participa indudablemente en la hematopoyesis y que su secreción está íntimamente bajo la influencia de la estimulación vagal.

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I communication 6

Eosinophils in the Blood of Tuberculous Patients

S LRDSTEIN J A AGULRO and A PALATANO*

Eosinophile counts after adrenalin injection were normal in 96% of tuberculous patients including those with suprarenal amyloidosis Postmortem examination of the suprarenal glands of 200 tuberculous patients showed normal glands in 85% amyloidosis in 8% and tuberculous lesions in 7% In none of these cases were the lesions sufficiently large or bilateral to have produced an Addison syndrome during life These functional and anatomic studies do not confirm the often so called functional insufficiency of tuberculous disease

LOS EOSINÓFILOS DE LA SANGRE EN LOS ENFERMOS TUBERCULOSOS

Los autores estudiaron en un elevado numero de pacientes tuberculosos a) el numero de eosinófilos de la sangre b) las modificaciones post adrenalinicas de los mismos (Recant Thorn) c) los efectos del tratamiento específico antituberculoso (estreptomicina tiosemicarbazona hidrazida del ácido isonicotínico)

Estos estudios se llevaron a cabo en las distintas formas de tuberculosis pulmonar y en sus complicaciones (incluyendo la amiloidosis generalizada y corticosuprarrenal)

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I communication 7

Panhypopituitarism and Diabetes Insipidus Hormonal Effects on Circulating Eosinophils and the Excretion of 17 Ketosteroids and Estrogens

LUIS J BERGNA FIDEL SCHAPOSNIK and ANGLI GUTIERREZ*

A case of panhypopituitarism associated with diabetes insipidus in a male 36 years old probably due to a craniopharyngioma is reported

The effects of ACTH adrenalin antidiuretic hormone serum gonadotropins testosterone de oxycorticosterone and desiccated thyroid on the circulating eosinphils urinary 17 ketosteroids and substances of estrogenic activity have been studied and the results discussed

PANHIPOPITUITARISMO Y DIABETES INSIPIDA EFECTOS HORMONALES SOBRE LOS EOSINOFILOS CIRCULANTES Y LA EXCRECION URINARIA DE 17 CETOESTEROIDES Y ESTROGENOS

Se relata un caso de panhipopituitarismo asociado a una diabetes insipida en un hombre de 36 años debido probablemente a un craniofaringioma

Se estudiaron los efectos inducidos por la adrenocórticotropina adrenalina hormona antidiurética gonadotropina sérica testosterona desoxicorticosterona y tiroides desecada sobre los eosinófilos circulantes los 17 cetoesteroides neutros urinarios y esteroides ácidos de actividad estrogenica discutién los los resultados logrados

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I communication 5

Study of the Erythropoietic Effect of Repeated Injections of Plasma Obtained from Rabbits Rendered Anemic by Bleeding

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It has been demonstrated that the injection of plasma from rabbits anemiated by bleeding produces an increase of reticulocytes in normal rabbits. In the present work it is found that the repeated injection of plasma from rabbits anemiated by bleeding produces a significant increase of the reticulocytes, red cells and hemoglobin in the normal rabbits.

After finishing the series of injections the red cell and hemoglobin values remained without variation in the control animals. The contrary occurred in the experimental group: a sustained increase of the red cells and hemoglobin was observed that reached its maximum on the tenth day. The maximum increase was 20% for red cells and 10% for hemoglobin. The unequal increase of red cells and hemoglobin would indicate that this result is not due to hemoconcentration. The hemoglobin and red cells value returned to normal 20 days after finishing the series of injections.

Plasma from animals kept in altitude climate has erythropoietic effect (Bonsdorf, Reissman). This fact together with the results of our experiments would indicate that a humoral factor intervenes in the stimulation of the erythropoiesis by hemorrhage or altitude climates.

Estudio del Efecto Eritropoyético de Inyecciones Repetidas de Plasma de Conejos Anemizados por Sangría en Conejos Normales

Se ha demostrado que la inyección de plasma de conejo anemizado por sangría produce un aumento de los reticulocitos en conejos normales (Krumdieck).

En el presente trabajo se comprueba que la inyección repetida de plasma de conejo anemizado por hemorragia produce aumento significativo de los reticulocitos, de los glóbulos rojos y de la hemoglobina en los conejos normales.

Después de finalizada la serie de inyecciones se mantuvieron sin variación los valores de los globulos rojos y de la hemoglobina en los animales controles. Por el contrario en el grupo experimental se observó un alza sostenida de los glóbulos rojos y de la hemoglobina que llegó a su máximo el día 10. El aumento máximo fué de un 20% para los globulos rojos y de un 10% para la hemoglobina. Este incremento desigual de los glóbulos rojos y de la hemoglobina indicaría que este resultado no se puede atribuir a una hemoconcentración. Los valores de la hemoglobina y de los globulos rojos se normalizaron 20 días después de terminada la serie de inyecciones.

El plasma proveniente de animales mantenidos en clima de alturas tiene accion eritropoyética (Bonsdorf, Reissman). Este hecho unido a los resultados de nuestros experimentos indica que es muy probable la intervención de un factor humoral en la estimulación de la eritropoyesis por hemorragia o por clima de altura.

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This discovery tends to suggest the existence of a neuro humoral mechanism capable of explaining certain experimental hemolyses and perhaps even certain hemolyses occurring in the human being These hemolyses might be due to the production and/or liberation (at the level of C N S) of an hemolytic substance causing destruction of hematies (lyso lecithine lecithina is) Thus in anesthetized animals benzene would no longer act upon the C N S and consequently hemolysis would not occur

Narcosis does *not* prevent hemolysis provoked by endovenous injections of saponine or ammonium since in this case the chemical agents work hemolytically and directly upon the membrane of the erythrocytes without any intervention of the nervous system

The protective action of chloroform can be observed equally well against other agents (cyclo hexene carbon bisulfide etc) although it is less evident than in cases of hemolysis by benzene

After tying up the arterial trunks emerging from the aortic crook (in order to separate the encephalic circulation from that of the rest of the body) towards the animal s heart even an hemolytic dosis of benzene injected will not produce hemolysis which proves that intervention of the C N S is required for benzene hemolysis to take place

The injection of relatively high doses of benzene into the femoral artery after tying up the crural vein (and thus isolating the leg from the rest of the body) does not provoke hemolysis of the blood tested from this vein which is a clear proof against direct action of benzene on the circulating hematids

Accion Hemolitica del Benceno y Otros Agentes Quimicos Su Prevencion por los Anestesicos

El benceno y sus homologos tolueno y xileno inyectados puros sin vehículo alguno en la vena del conejo provocan la muerte del animal con hemólisis intensa A igualdad de dosis el tolueno es menos tóxico que el benceno y el xileno menos que el tolueno La adición de lecitina o colesterol no modifica la accion hemolitica del benceno mientras que el colesterol protege claramente contra la hemolisis por saponina La adición de aceite de olivas al benceno impide la hemólisis y muerte del animal probablemente por acción directa del aceite sobre el benceno

La narcosis con cloroformo o éter por vía inhalatoria o con cloral o paraldehido por vía intraperitoneal impide la muerte del animal y la hemolisis por benceno en el 100% de los casos mientras que con pentotal y xilocaína la protección contra la hemólisis alcanza al 60–70% de los animales tratados Este hallazgo permite sugerir la existencia de un mecanismo neurohumoral para explicar ciertas hemólisis experimentales y quizá también ciertas hemólisis que ocurren en el hombre Tales hemólisis serían debidas a la producción y/o liberacion a nivel del S N C de una sustancia hemolitica que actuaría sobre los hematíes provocando su lisis (lisolecitina lecitinasa?) En los animales anestesiados el benceno no actuaría sobre el S N C y en consecuencia la hemolisis no se produce La narcosis no previene la hemólisis provocada por la inyeccion intravenosa de saponina o amoníaco por tratarse en estos casos de agentes químicos que ejercen su acción hemolítica en forma directa sobre la membrana del eritrocito sin intervención del sistema nervioso La acción protectora del cloroformo se comprueba asimismo frente a otros agentes (ciclohexeno sulfuro de carbono etc) aunque de manera menos evidente que en el caso de la hemólisis benzénica

La inyección de una dosis hemolítica de benceno después de la ligadura de los troncos arteriales que emergen del cayado de la aorta (separando así la circulación cefálica de la del resto del cuerpo) hacia el corazón del animal no produce hemólisis lo que prueba que la intervención del S N C es necesaria para que la hemólisis bencénica tenga lugar La inyección de dosis relativamente altas de benceno en la arteria femoral después de la ligadura de la vena femoral (aislando así la pata del resto del cuerpo) no provoca la hemólisis de la sangre recogida de esta vena lo que depone claramente en contra de una acción directa del benceno sobre los hematíes circulantes

I communication 8

Thrombopenic Purpura Appearing after Cranial Traumatism

T C MINNHAAR*

An always healthy two year old child eutrophic of healthy parents falls and hits edge of marble steps with forehead February 11th 1947 Large hematoma on spot of traumatism sanguineous suffusion of eyelids of both eyes and on the 3rd day petecchial rash appears all over the body (skin and mucous membranes)

No toxic or infectious antecedents normal alimentation Discrete anemia with normal leukocyte count absence of platelets lengthened bleeding time normal coagulation sign of Rumpel Leede intensely positive Recovers in 10 days with transfusions vitamin C hepatic extracts and coagulants

A probable neurogenic etiology is assigned to this purpura after reviewing numerous diagnostic possibilities

PURPURA TROMBOPÉNICA APARECIDA DESPUÉS DE TRAUMATISMO DE CRÁNEO

Niño de dos años de edad eutrófico de padres sanos que ha sido siempre sano cae y golpea con la frente en el borde de un escalón de mármol el 11 de Febrero de 1947 Gran hematoma en el sitio traumatizado sufusión sanguínea en los párpados de ambos ojos y al tercer día erupción petequial en todo el cuerpo (piel y mucosas) Falta absoluta de todo antecedente tóxico e infeccioso alimentación normal

Discreta anemia con formula leucocitaria normal ausencia de plaquetas Tiempo de sangría prolongado coagulacion normal signo del lazo positivo intenso Se recupera en 10 días con transfusiones vitamina C extractos hepáticos y coagulantes

Se asigna una probable etiología neurogena a esta purpura después de pasar en revista numerosas posibilidades diagnosticas

Rosario Argentina

I communication 9

Hemolytic Action of Benzene and Other Chemical Agents Its Prevention by Anesthetics

LEON BRAILR MARIO FRANCONE and A CALABRESE*

Benzene and its homologues toluene and xylene injected pure without any vehicle into a rabbit s vein provokes death of the animal through intense hemolysis In equal dosis toluene is less toxic than benzene and xylene even less so than toluene Added lecithine or cholesterol will not modify the hemolytic action of benzene while cholesterol clearly protects against hemolysis by saponine An addition of olive oil to benzene prevents hemolysis and death of the animal presumably because the oil acts directly on the benzene

Narco is by inhalation of chloroform or ether as well as by intraperitoneal injection of chloral or paraldehyde will prevent death of the animals and hemolysis by benzene in 100% of the cases while a treatment with pentothal and xylocaine will protect only about 60–70% of the subjects

Ministerio de Salud Publica Buenos Aires Argentina

TABLE 2—*The Effect of Various Materials on the Number of Circulating Eosinophils in Adrenalectomized Mice Pretreated with Epinephrine*

St[illegible] [illegible]	Number of M	Material Injected	Average Eosinophil Response (3 hour)
Co BR/cd	23	µg Compound F	79 ± 2 2
	21	3 µg Compound F	60 ± 5 0
	14	1 µg Compound F	13 ± 5 ?
	4	Insulin (2 or 3 Units Squibb)	2
BBF₁	4	2 mg Histamine Diphosphate (Mead's Viscosterol)	18 (toxic)
	6	0 ? to 2 mg Heparin (Hynson Wescott Dunning)	7
	6	0 03 cc Irradiated Ergosterol (Mead's Viscosterol)	0
	6	100 µg Androsterone	0
	4	100 µg Testosterone	0
	3	100 µg Dehydroisoandrosterone	0
	5	100 µg Progesterone	0
	3	50 µg Alpha estradiol	0
	5	0 5 mg ACTH (Armour #125 105I)	0
	3	1 0 mg ACTH (Armour #125 105R)	0
	12	0 5 to 2 cc human urine prior to injection of ACTH	2 ± ? 1
	8	0 5 cc human urine during ACTH therapy	57 ± 6 4
	6	Chloroform extract of above urine equivalent to 0 5cc	39 ± 8 2

TABLE 3

EOSINOPHIL ASSAY FOR CORTISONE ACETATE

THEORETICAL POTENCY 100%

N	COMPOUND	RATIO OF DOSES µg	b SLOPE	S D	λ	POTENCY RATIO	S E %	t SLOPE
10	CORTISONE ACETATE	6/3	−81 3	6 7	0 206	98	6	0 569
6		6/3	88 5	18 3	0 207	97	14 6	482

ION 00 0

TABLE 4

COMPARISON OF BBF AND 129 MICE FOR ASSAY OF ACTH

MICE COMPARISON	N	RATIO OF DOSES OF ACTH I U	b SLOPE	S D	λ	POTENCY RATIO	S E %	t SLOPE
BBF BBF	6	2	−160	53 3	0 33	98	30	13
BBF vs 29		2	−12	20 9	0 6	96	1	.56

The statistical analysis of our data was performed by Dr R I Dorfman and is shown in table 3

The assay of ACTH may be performed in intact or hypophysectomized mice The material to be assayed is suspended in 0 25 ml gelatine or 0 05 ml sesame oil The mice are isolated and kept undisturbed prior to and following the injection of ACTH Eosinophil counts are performed in the routine manner immediately prior to and 24 hours after the injection of

I communication 10

The Eosinophil Assay of Adrenal Cortical Hormones and ACTH

R S SPEIRS*

Various types of stress or hormone treatment have been found to produce a decrease in eosinophils in circulating blood Removal of the adrenal cortex prevented these changes and single injections of adrenal cortical materials produced identical eosinopenic responses (Thorn et al 1948 Speirs and Meyer 1949) These observations led to the suggestion that the decrease in circulating eosinophils might be used to quantitatively estimate the amount of adrenal cortical hormone injected into adrenalectomized rats and mice (Speirs and Meyer 1949 and 1951 Rosemberg and Lewis 1950) It was further adapted to the assay of urinary corticoids (Speirs Wragg Bonner and Homburger 1951) and ACTH (Speirs 1952)

The eosinophil assay has been found to be highly sensitive and specific for the 11 oxy *corticosteroid hormones It is performed as follows*

1 Male mice weighing 20 to 25 grams are adrenalectomized in a one step operation and 15 mgm pellets of desoxycorticosteroid acetate are implanted subcutaneously

2 Three or more days post operatively the mice receive a subcutaneous injection of 20 micrograms of epinephrine and the material to be assayed is injected 4 hours later

3 Eosinophil counts are taken immediately prior to and 3 hours following the injection of the assay material The percent decrease in the number of eosinophils during this period is correlated with the quantity of 11 oxycorticosteroid hormones injected

The type of mouse used in these assays was found to be important Strains related to the C57BR or C57BL were found to be similar in their responses and sensitive to as little as 1 microgram of cortisone acetate Other strains such as the CFCW 129 DBA and STR are not nearly as sensitive (See table 1)

TABLE 1 —*Relative Responsivity of Various Strains of Mice (Response = % decrease) (CFCW Mice = 100%)*

Strain	Number of Mice	Slope	Dosage Range Cortisone Acetate	Relative Response	Error Range 1 S E σ	Error Range 2 S E σ	t [illegible] p
CFCW	41	−55 42	3 6 12 18	100			—
129	30	−36 88	3 6 12 24 96	61	−25 +33	−43 + 6	1 507
C57 Black	22	−44 56	1 3 6 12	296	−26 +36	−46 +85	0 199
BBF_1	18	−53 95	1 3 6	495	−22 +29	−40 +67	0 068

Figure 1 illustrates the results obtained when synthetic adrenal cortical hormones are assayed We have injected many different sex hormones oils and non adrenal sterols and to date the only materials which produce the eosinopenic response in pretreated adrenalectomized mice are the corticosteroids of the adrenal cortex This is summarized in table 2

* R B Jackson Memorial Laboratory Bar Harbor Maine U S A

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Strain of Mice	Number of Mice	Material Injected	Av. Eosinophil Response (3 hours)
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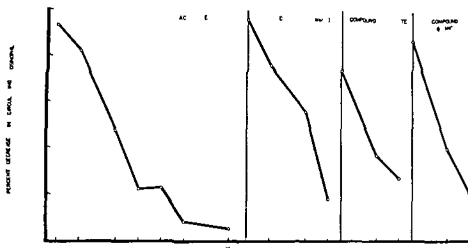
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LOG DOSE RESPONSE OF EOSINOPHILS TO VARIOUS SYNTHETIC ADRENAL CORTICAL HORMONES

Fig 1

ACTH The percent decrease in the circulating eosinophils over the 24 hour period is proportional to the amount of ACTH injected The statistical analysis of data obtained from this assay is shown in table 4

El Ensayo de Hormonas Corticales de ACTH con la Prueba de la Disminucion de los Eosinofilos

La disminucion de los eosinofilos circulantes puede ser usada para determinar cuantitativamente la cantidad de hormona córtico adrenal inyectada en las ratas y ratones suprarrenalectomizados La prueba es muy sensible y específica de los 11 oxicorticosteroides

Tiene importancia la cepa de ratones usada por su diferente sensibilidad El ensayo de la ACTH puede ser realizado en animales normales o hipofisectomizados y la disminucion de los eosinofilos es proporcional a la cantidad de ACTH inyectada

PART II

Histochemistry and Cellular Ultrastructure

Histoquímica y Ultraestructura Celular

Electron Microscope Studies of Circulating Blood Cells

E DE ROBERTIS*

IN THE last few years electron microscope analysis of the circulating blood elements has become increasingly important Great advances were made in hematology, as well as in other biological fields thanks to the development of new techniques for the suitable preparation of the materials to be observed with the electron microscope Circulating blood provides us with a very favorable material since its various components are dispersed in a liquid medium They do not require thus the employment of special fragmentation techniques However with the exception of the macromolecular components of blood plasma such as fibrinogen fibrin and other proteins the circulating blood cells are completely opaque to the electron beam This difficulty has been partly overcome thanks to recent technical advances which make these elements partially transparent to the electron beam Different procedures have been employed depending on the elements considered i e erythrocytes leucocytes or platelets and we shall describe them briefly in the corresponding sections

This report is not intended to give an exhaustive review of the literature and of the different techniques employed up to the present moment We shall refer mainly to the methods adapted or developed in our Department of Cell Ultrastructure for the study of erythrocytes leucocytes platelets and blood proteins When dealing with the results obtained we shall also give preferential attention to the problems we have studied or which are being studied at present

We are convinced of the fact that thanks to the advances already done ultrastructure has a very promising future in the field of hematology It is very likely that the electron microscope examination of the circulating blood elements or of material obtained by biopsy will soon become a routine procedure for the clinical study and diagnosis of certain diseases The data thus obtained might be as useful or even more than those furnished by cytological studies with the optical microscope

The original data presented in this report were obtained with the collaboration of B Epstein P Paseyro Magdalena Reissig A M Fonseca C M Franchi and M Podolski and the proper acknowledgment will be found in the different sections of the paper and in the references

Ultrastructure of Erythrocytes

Because of their hemoglobin content intact erythrocytes are completely opaque to the electron beam However after hemolysis as shown first by Wolpers

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This work has been supported by a grant from the Rockefeller Foundation

Thanks are given to Prof W H Seegers for providing the thrombin mentioned in the section on The Action of Thrombin on the Ultrastructure of Platelets

(1941) and by Wolpers and Zuckau (1942) the remaining membrane appears as a hollow baloon folded on the supporting film These and other results tend to show that human erythrocytes do not possess an internal structure or stroma and that they can be considered as made up of a membrane containing a highly concentrated colloidal solution of hemoglobin This membrane would be constituted by a protein meshwork whose pores are filled by the lipid component According to Wolpers these holes filled with lipids would be responsible for some of the permeability mechanisms If the membrane is treated with lipid solvents *the protein network becomes more evident A theory of permeability based on* the presence of phospholipids in the pores of the membrane has been recently developed by Parpart (1952)

A systematic study of hemolysis as seen under the electron microscope has been done by Lindemann (1949) and by Bessis and Bricka (1950) These last authors have also studied the process of agglutination and change of shape of the erythrocytes The surface of the erythrocyte in relation to hemolysis has been recently studied by Latta (1952)

Reticulocytes have been studied by several authors Bernhardt and col (1949) Braunsteiner and Bernhardt (1950) and Peters and Wigand (1950) are among the more recent ones The granular filamentous substance has generally been seen to appear as vesicles from 0 2 to 0 7 μ in diameter arranged in groups or chains

We have started the study of hematological material in 1950 The work done covers mainly the relation existing between erythrocytes and certain intraglobular viruses and parasites producing diseases of considerable importance in veterinary medicine We have also begun to study the human erythrocyte both in normal and pathological conditions

Electron Microscope Study of Red Cell Membranes after Experimental Infection with Foot and Mouth Disease Virus

The red cell agglutination phenomenon by influenza virus first reported by Hirst (1941) and McClelland and Hare (1941) has been used in the study of several viruses with the electron microscope The initial phase of this phenomenon consists in the virus adsorption on the membrane surface The presence of virus particles can thus be demonstrated by studying the membranes under the electron microscope Using laked human erythrocytes and specially chicken erythrocytes Heinmets (1948) was able to study the influenza virus Dawson and Elford (1949) greatly improved this technique and studied the adsorption *of influenza A and B virus fowl plague Newcastle disease and epidemic parotiditis* It was thus found that the concentration of virus particles on the membrane per unit area varied according to the virus and red cell concentration the time of exposure to the virus and the temperature it was also found to be characteristic for the different virus species We developed in our laboratory a very simple technique for laking a blood smear directly on the slide By these means it was possible to observe the red cell membranes under the electron microscope and we were able to undertake the direct study of the virus cell relationship in certain animal infections As the existing literature furnished us

with data indicating the possibility of a relationship between the erythrocytes and the foot and mouth disease virus during the generalization period of the disease (Vallé and Carré (1921) Graub Zschokke and Saxer (1939) we decided with Drs Epstein and Fonseca (1951) to study red cell membranes of guinea pig in various stages after inoculation with the type O of foot and mouth disease virus

The technique employed has been denominated by us of *double hemolysis* It consists in the taking of the blood smear in distilled water after which we cover it with a parlodion film and float it on water The material is observed either directly or after shadow-casting

The advantage of this technique is that aside from its simplicity and quickness it enables us to obtain absolutely flat red cell ghosts on the film that generally do not show folds which might interfere with observation (Fig 1) Dense masses constituted by clusters of smaller particles are seen in the red cell membranes of guinea pigs after 24 to 72 hours inoculation with the virus (Fig 2) The number and size of these particles increases during this period and they can no longer be seen after 92 hours This has been related to the fact that red blood cells obtained during the acute period of the disease and washed in saline are infective and that with the decline of the general symptoms i e when the particles with the erythrocytes disappear no infection occurs These facts may be suggestive of a direct relationship between the presence of the infective virus within the erythrocyte and the masses of particles seen in the membranes under the electron microscope These masses resemble the virus inclusions found in many *virus diseases which are considered as formed by groups or colonies of* elementary bodies The virus host cell relationship in our case is different from the above mentioned simple process of virus adsorption by the red cell membrane The difference found tends to indicate that the inclusions or colonies represent an active phase in the process of virus reproduction in the erythrocyte membrane during the generalization period of the disease The appearance and disappearance of the inclusions would be likewise related to the vital cycle of the virus within the host-cell

Study of Anaplasmosis in Bovine Erythrocytes with the Electron Microscope

With Epstein we applied the above mentioned double hemolysis technique to the study of certain intraglobular parasites of great interest in Veterinary Medicine (De Robertis and Epstein 1951) We are referring to pyroplasmosis and specially to anaplasmosis which causes in bovine a severe anemia that may lead to death of the animals The anaplasma marginalis Theiler (1909) is very appropriate for electron microscope study since its diameter is of only 0 4 to 1 μ It has been generally described as a rounded marginal body made up of a single Feulgen positive chromatin mass lacking cytoplasm It has been assumed that the parasite multiplies by binary division The electron microscope study reveals two very interesting characteristics First that the parasites which appear as single masses under the optical microscope are generally formed by an

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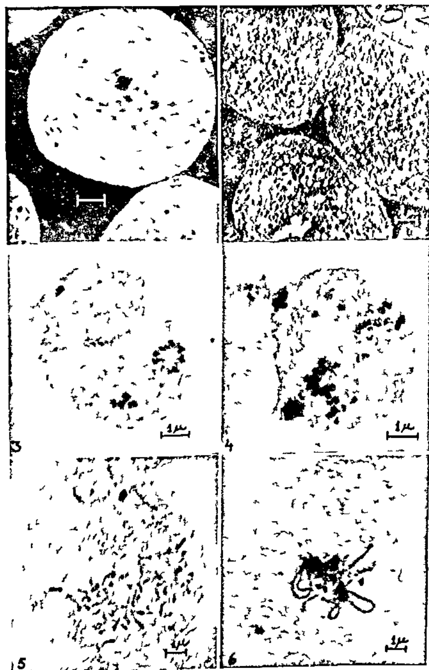


FIG 1.—Red cell membranes from a normal human 8 500 × (*Note* All illustrations have been reduced 25 per cent in reproduction Figs 1, 2, 5 and 6 are from preparations shadowed with palladium at 11.)

FIG 2.—Red cell membranes from a guinea pig 2 hours after inoculation with the foot and mouth disease virus Round masses mainly disposed in single lines protrude over the surface 6 500 ×

FIG 3.—Red cell membrane of a calf infected with *Anaplasma marginale* The marginal parasite shows round bodies at the periphery In addition there are 5 elementary bodies dispersed within the erythrocyte 9 400 ×

undivided central mass surrounded by peripheral rounded bodies 170 to 200 mμ in diameter (Fig 3) In some cases all the bulk of the parasite seems to be formed by tightly clumped elementary bodies Second that aside from the bodies found in the parasite itself a great number of them are scattered throughout the erythrocyte Most of these are submicroscopic (Fig 4) and they certainly could not be seen under the optical microscope A few larger ones are in the limit resolving power of the optical microscope and they could easily be mistaken with granular basophile material found in some normal and pathological erythrocytes

These two facts suggest that besides the marginal bodies shown by the optical microscope smaller parasitic units exist The presence of these submicroscopic units could explain some of the clinical characteristics of the disease such as the fact that the infective agent persists in the circulating blood after the disappearance of the general symptoms At this stage the number of marginal bodies is very scarce and sometimes they are not even seen at all

These observations are suggestive of the fact that instead of the generally accepted binary fission multiple division of the parasite takes place The morphological entities represented by the classical marginal bodies and by the submicroscopic elementary bodies seen with the electron microscope within the parasite as well as out of it probably represent different stages of the vital cycle of the anaplasma in the circulating blood

ULTRASTRUCTURE OF LEUCOCYTES

Considerable advances have been made in the last few years in the electron microscopy of leucocytes We shall consider them briefly since we have not made a special study of them

It has been known for some time that various cells possess the property of flattening out on a glass surface thus becoming thin enough to be partially transparent to the electron beam This property was known for the circulating blood cells of lower animals such as amibocytes coano leucocytes etc and also for cells cultured in vitro

Regarding the leucocytes of higher animals it has already been observed with the optical microscope that they adhere to glass surfaces in small number Jolly (1923) Fenn (1923) Ebergengy (1924) von Philipsborn (1934) and Veplens (1945) It was also known that in certain infectious diseases and particularly in pneumonia they became adherent in much larger number In 1949 Bessis and Bricka demonstrated that adhesion and flattening of the polymorphonuclear leucocytes of human blood could easily be obtained when employing a plastic membrane such as silicon plexiglass parlodion cellophane formvar etc The authors isolated the white cells by differential centrifugation of the blood and deposited them on the plastic The technique was simplified by Bernhardt et

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FIG 4 —The same as in fig 3 In addition to an undivided mass (lower left) a large number of bodies are dispersed throughout the erythrocyte 9 700 ×

FIG 5 —Red cell membrane of a human with acute leukemia Dense material disposed in round masses rods and clubs are seen 6 500 ×

FIG 6 —The same as Fig 5 Dense long and wavy filaments are seen 6 700 ×

al in 1950 The authors immerse glass slides coated with a *formvar or parlodion* film in heparinated blood for 15 to 30 minutes at 37°C Studies of this material with the optical microscope after staining in the usual way, revealed that leucocytes became adherent to the film in the following proportion polymorphonuclear leucocytes 90 per cent, monocytes 6 per cent, lymphocytes 4 per cent According to Bessis (1951) the granulocytes are the only ones that flatten out but according to Bernhardt et al (1950) this also happens with the monocytes and lymphocytes if the temperature is kept between 37 C and 40°C When the granulocytes become flattened they show three zones (nuclear, perinuclear and hyaloplasmic) having different thickness and permeability to the electron beam (Fig 7) The nuclear zone is generally too dense and no details can be distinguished in it The perinuclear zone contains the specific granules and the mitochondria which appear as thin rods The eosinophils have very dense *spherical or ovoidal granules Bessis has made a study of the granules* employing not only the above mentioned technique, but also after cell destruction and by means of replicas The peripheral hyaloplasmic zone generally lacks specific granules According to Bernhardt and coll it appears as a finely granular network This hyaloplasm gives rise to thin and long pseudopodia They generally appear in a certain zone of the cell edge and extend away from it until they reach a distance equivalent to 1 to 4 cell diameters showing frequent branching

Lymphocytes usually emit thin pseudopodia all around the cell Monocytes generally lack true pseudopodia and they show a sinuous undulating membrane, while the perinuclear cytoplasm contains a great number of mitochondria (For further information see also Bernhardt et al 1950 Bessis 1951 Bessis 1952)

ULTRASTRUCTURE OF LEUKEMIC CELLS THE ULTRACHONDRIOME

In 1951 Bernhardt et al published an interesting paper about the leukemic cells of the circulating blood with special reference to acute leukemia They observed that these cells are polymorphic when extended and that they lack the exuberant pseudopodia of normal leucocytes The ground cytoplasm does not show special differences with the normal The nuclear zone is generally less dense The most characteristic changes occur in the type of the granules The e are 1) specific granules and mitochondria similar to tho e seen in the normal cells and 2) smaller granules having high electron density At the beginning this dense material was thought to be *characteristic of the leukemic cells* These granules are either isolated or arranged in pairs as indicating a multiplication process or forming groups or chains composed of 5 to 10 elements The diameter of the e bodies varies between 40 and 170 mμ Filamentous and granulofilamentous elements of varied shapes showing all kinds of transition types have also been seen The authors claim to have found similar elements in cells from different tumoral and inflammatory processes and also in very rare instances in monocytes from normal subjects These facts together with the high degree of polymorphism shown by this material and the existence of a continuous series of transitional forms between it *and the mitochondria made them discard*

the hypothesis that they could be virus material. They considered rather that they represent a precursor of the chondriome and on this account they named it ultrachondriome. It is interesting to bear in mind that similar elements have been found by Porter and Thompson (1947) in cultured sarcomatous cells, by Dalton et al. (1949) in cellular fragments from different tumors and by Porter (1951) in growing embryonic cells. According to Porter this material would be endowed with the property of multiplication and is related to the process of protoplasmic growth and synthesis (see also Bessis (1951) and Oberling et al. 1951).

Observation of Erythrocytes in a Case of Acute Leukemia

In relation with the above mentioned papers of the French authors we wish to show some images obtained in red cell membranes from a case of myeloid leukemia that underwent an acute course after radiotherapic treatment. These observations have only a casuistic interest since the images could be seen in great number only in one of the six cases studied and only a few of these images could be seen in another two. In the case we are presenting now studied in collaboration with Dr. Paseyro we examined smears obtained in three different stages of the disease and it was found that the affected erythrocytes increased in number up to the last observation prior to the patient's death.

As shown in the figures the membranes show the presence of a material disposed in granules, rounded masses and specially in rods, clubs of filaments (Fig. 5). These filaments are sometimes long and wavy (Fig. 6). The considerable number of elements of these types found, which sometimes covers the membrane partially, makes us think that it might be an actively growing material. All these elements, even the filamentous ones whose diameter varies between 100–200 mμ appear as formed by dense granules having a diameter of 100 mμ. We have to point out that granulo filamentous elements similar to those described by Bernhardt and coll. were also found in the leucocytes of this case.

If these observations receive confirmation when examining other cases, the interpretation of their nature would be a difficult one. The hypothesis of the existence of an ultrachondriome in a cell normally lacking it would be rather untenable and we would probably have to think of an agent undergoing multiplication within the red cells. It might happen, of course, that in this particular case this agent has no relationship whatsoever with leukemia and it is only coexistent element. At any rate we think that it would be very interesting to repeat these observations particularly if a biological investigation of the material could be done.

Ultrastructure of Blood Platelets

Blood platelets are more adequate than any other of the circulating blood elements for electron microscope observation. This is due to the small diameter they have in human and mammalian blood and to the fact that they become extended and flattened with great ease when leaving the blood stream. Wolpers and Ruska (1939) examining blood fixed in osmic acid found that the platelets

Fig. 7-8

appear as spherical completely opaque masses Their internal structure stands out after a short fixation with osmic acid followed by immediate washing and extraction in distilled water

Under these circumstances platelets become swollen showing their two main components The hyalomere which appears as a finely reticular protoplasm and the chromomere or granulomere constituted by granules 110 to 140 mμ in diameter having a high electron density The number of these granules varies from 20 to 120 It is interesting to point out that with the optical microscope only 10 to 20 granules can be seen According to Wolpers in some cases and particularly in thrombocytopenia the granules are either very scarce or absent It would be very interesting to repeat this observation applying modern techniques

Bessis and Burstein (1948) in one of the most recent electron microscope studies describe the different shapes assumed by the platelets when they leave the blood stream and become extended on a surface such as a plastic film The technique consisted in centrifuging slightly the heparinated blood after which a drop of plasma rich in platelets is deposited on a plastic coated glass slide In these conditions the platelets undergo a series of changes in shape already described by Fonio (1942) by examination with the ultramicroscope These different forms are

1 The circulating spherical form already described

2 The dendritic form in which the hyalomere shows processes extending 3 to 15 μ away from the platelet body The thrombocyte assumes the shape of a polyp a starfish a spermatozoon etc , according to the number and length of the processes In this stage the central zone of the platelet appears completely opaque and it is impossible to distinguish the chromomere (Fig 8)

3 The transitional forms After the central zone of the platelet comes in contact with the slide it begins to flatten and becomes extended The dendritic processes shorten and widen at their base They thus fuse with the protoplasm of the hyalomere which constitutes a sort of undulating membrane

4 The extended forms The final stage of this process which may take place within the very short period of 5 to 30 minutes after placing the platelets on the film is the extension and flattening of the platelet These extended forms assume a round ovoidal or polygonal shape with clear cut edges devoid of processes or having only a few tips corresponding to rests of pseudopodia Their size is variable but they can become 3 to 5 times larger than in the circulating form

The flattening of the cytoplasm makes it possible for the internal structure to be seen under the electron microscope The chromomere appears as a zone of greater electron density with very dense spherical or ovoidal granules The diameter of these granules is variable oscillating between 60 to 150 mμ They are disposed in groups sometimes in rows according to the shape and orientation of the hyalomere It seems very likely that these granules (whose number varies

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Fig 7 —A human neutrophile leucocyte extended on a parlodion film and two platelets Osmic acid fixation 7 000 ×

Fig 8 – Normal human platelets on a parlodion film *d* dendritic *tr* transitional and *e* extended forms of platelets Osmic acid fixation 400 ×

from 10 to 50 according to Bessis and Burstein), are surrounded by an amorphous and dense material which in the less extended forms covers and hides them (*Fig 8*)

The ultrastructure of the hyalomere has been the object of a special study by Bessis and Bricka in 1948 It has a variable appearance according to the fixative used With osmic acid fixation it appears as a hyaline structureless material with formalin it shows an alveolar structure and it appears as fibrillar when using absolute alcohol The fibers would have a circular or radial disposition According to these authors the fibrils would be constituted by small spherules 50 to 75 mμ in diameter linked by thin bridges

In our Department we have repeated the observations of the above mentioned authors and we have also studied

THE ACTION OF THROMBIN ON THE ULTRASTRUCTURE OF PLATELETS

Quick has analyzed in his recent book (1951) the role of platelets in blood clotting from the historical viewpoint This role had already been recognized in the last century by Hayem Kemp and Bizzozero, who stated that the fibrin network does not make its appearance unless the platelets become altered thus providing a substance which the plasma normally lacks and making clotting possible In 1904 Burker *mentioned the role of the platelets by saying that* every time coagulation is prevented the disintegration of platelets is prevented This hypothesis was reinforced by the experiences of Delezenne and of Tait and Green who prevented the clotting of fowl blood by separating the thrombocytes According to Quick (1951) platelets remain intact when placed in decalcified heparinized or hemophilic plasma or if the plasma has a marked hypoprothrombinemia These four types of plasma have one thing in common the lack of thrombin According to this author thrombin would be directly responsible for the labilization and lysis of the platelets He also mentions papers by Fonio (1940) and Zatti (1948) who state that thrombin acts on the thrombocytes causing their agglutination This hypothesis together with previous work made it very promising to study the action of thrombin on the blood platelets under the electron microscope preventing if possible the process of clotting In this case if platelet lysis occurred it would be a direct effect of thrombin action not a result of the blood clotting process The experiments done in collaboration with Drs P Paseyro and Magdalena Reissig prove in a definite way that thrombin has a lytic action on blood platelets

The experimental work consisted in obtaining platelets from normal human *blood by using the technique described by Bernhardt et al (1951) for leucocytes* and platelets and to treat them with thrombin Slide fragments coated with a parlodion film are immersed in tubes containing heparinized blood and then kept at 35°C for 15 to 20 minutes This period is enough for a considerable number of platelets and a few leucocytes to become attached to the slide The slide fragments are then washed in Tyrode solution kept at 35°C until all the red cells and the fibrinogen have been washed out (4 or 5 changes of the Tyrode *solution are generally sufficient*) The slides are then immersed in the thrombin

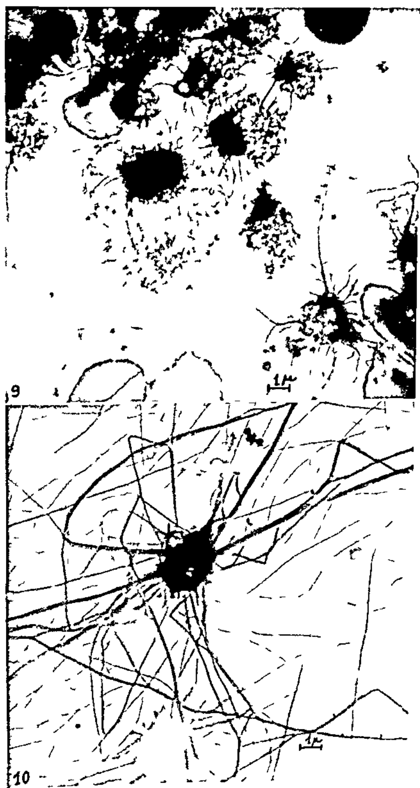
solution Parke Davis bovine thrombin for local use containing 1000 units per ml was used This sample was diluted in Tyrode solution to obtain a final concentration of 25 to 50 units per ml according to the determination of thrombin activity made applying the method proposed by Quick (1951) Positive results were obtained with thrombin of Parke Davis and we thought it interesting to repeat the experience using a highly purified thrombin sample prepared by Prof W H Seegers from Wayne University This thrombin is prepared by activating purified bovine prothrombin in concentrated sodium citrate solution and then lyophilized This sample was used at a final concentration of 0 25 mg per ml in Tyrode solution Its activity was found to be according to our determinations of 50 thrombin units per ml In further experiments concentrations of 2 5 5 20 and 50 units of thrombin per ml were used

In the first experiment the platelets were treated with Parke Davis thrombin at a concentration of 25 units per ml *during periods of time oscillating between* 5 to 30 minutes The progressive destruction of the platelets undergoing this treatment was thus demonstrated A second experiment was done to compare the action of Parke Davis thrombin with the purified sample of Seegers Both samples were made to act at a concentration of 50 units per ml during 20 minutes Platelets on which Tyrode solution and normal heparinated plasma had acted also during 20 minutes at 36°C were used as controls After this treatment the slides were washed in Tyrode solution and fixed in osmic acid vapors for 3 to 5 minutes

The thrombocytolytic effect of thrombin can be observed in Fig 9 It can be seen in the control experiment that most of the platelets belong to the extended or to the transitional form and that they are aggregated in clusters In the treated slides besides the normal extended platelets others can be seen whose protoplasm is undergoing disintegration in different degrees The phenomenon of lysis progresses from the peripheral to the central zone The hyalomere disintegrates giving rise to fibrillar elements apparently constituted by a row of microvesicles linked together In a more advanced stage of disintegration these fibers break up giving rise to groups of isolated microvesicles

The internal zone or granulomere turns into a rounded opaque amorphous mass where the granules are not clearly outlined The granulomere seems to undergo lysis at a relatively early stage since the above mentioned changes can be observed even before disintegration of the hyalomere takes place Finally opaque bodies and vesicles are left on the film as disintegration end products In the cases where a fibrin network is seen lysis of the platelets occurs in the same way Experiments with concentrations of thrombin as low as 2 5 units show a definite lytic action The number of lysed platelets is however proportional to the concentration of thrombin

The great sensitivity of platelets to small amounts of thrombin lead to the assumption that this substance besides acting as an enzyme in the fibrinogen fibrin reaction may also play a role in normal blood clotting It seems possible that in the normal clotting process as soon as a small amount of thrombin is produced in a definite locus an autocatalytic reaction takes place by means



FIGS 9–10

of which a certain number of platelets become more labile and undergo disintegration This process in turn will lead to the local production of more thrombin which will react with new platelets in a kind of chain reaction

Ultrastructure of the Blood Clotting Process

The complex chemical transformations involved in the process of blood clotting are finally expressed in macromolecular and cellular changes that can be followed under the electron microscope The best studied among these are the fibrin network and some of the stages in the conversion of fibrinogen into fibrin The information available on fibrinogen macromolecules and on the role of platelets in coagulation is on the contrary scarce and contradictory

The fibrin network produced during the clotting of whole plasma was studied by Wolpers and Ruska in 1939 These authors described it as formed by bundles of micelles running parallel to each other to form thicker trabecules joined by communicating bundles A cross striation having a period of 200 to 250 Å was found by them in clots from tuberculous meningitis This was the first periodic structure found in a fibrous protein It was later seen that this is a quite general property also present in other proteins From the existence of this regular structure it can be concluded that fibrin molecules not only have a parallel arrangement but that they also attain a high degree of lateral order and that they are in phase within the same fibril A cross striation with a period of 180 A was later found by Wolpers (1947) in blood fibrin These observations were simultaneously confirmed by Hawn and Porter (1947) in clots resulting from the interaction between thrombin and purified fibrinogen These authors *found in them a period of approximately 250 Å* Hall *studying a similar system* found a period measuring an average of 227 A and having an intermediate paler band located at about the middle of the macroperiod The stages in the change of fibrinogen into fibrin have been followed by Hall (1949) and by Porter and Hawn (1949)

These authors have nevertheless quite different views on the morphology of the fibrinogen molecule While Hall describes them as filaments 600 Å in length and 30 to 40 A in width Porter and Hawn consider them as made up of fine particles not showing any evidence of fibrilar structure

The first changes are observed already 15 seconds after mixing fibrinogen and thrombin They consist in an orientation of the particles into short chains and *small needle shaped structures According to Porter and Hawn* the fibrin fibrils would result from the longitudinal and lateral aggregation of these needle shaped fibrils resembling tactoids which gradually acquire the above mentioned striation

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Fig 9 —Platelets treated with 50 units of thrombin (Parke Davis) for 20 minutes Most of the platelets are in different stages of disintegration (see the control Fig 8) Osmic acid fixation 5 400 ×

Fig 10 —Blood clotting occurring spontaneously on the film of parlodion A submicroscopic fibrin network is being formed by lateral association of acicular tactoids Large fibrin fibrils converge and cross through a platelet which is in the process of disintegration At the bottom another extended platelet has no relationship with the fibrin Guinea pig blood , 000 ×

Our observations on the clotting process refer only to a few of its aspects In general we agree with Porter and Hawn, regarding the formation of spindle or needle shaped elementary fibrils that unite later to form thicker fibers with parallel edges

Braunsteiner and Febvre (1949) also confirmed these results for a system containing platelets These authors observed the close relationship existing between the platelets and the fibrin network Similar phenomena have been observed by us However not all the platelets appear to be in such a close relationship with the fibrin network In Fig 10 we can see that while one platelet is integrating the network, and appears as a nodal point where several fibers are crossing another one can be seen which is completely independent from the fibrin The platelet in connection with the fibrin is in the process of disintegration

Study of Fibrinogen and Carboxyhemoglobin Molecules

We have already mentioned the lack of agreement existing regarding the morphology of the fibrinogen molecule Now we shall mention the results obtained by means of a special technique developed in our laboratory for the study of fibrinogen and other macromolecules with the electron microscope This work was carried out with the assistance of C M Franchi and M Podolski (1951)

By means of this technique it is possible to demonstrate that at least in the conditions of preparation for electron microscopy the fibrinogen molecule is spherical or slightly ovoidal and not filamentous as has been claimed by Hall (1949)

Electron microscope examination of macromolecules is hindered by the tendency they have to clump on the supporting film When a drop of solution is deposited on the film regardless of its degree of dilution the macromolecules tend to clump on it when the solution becomes concentrated through desiccation and it is difficult to recognize the elementary units within these clumps

Besides if the dilution factor is very large the impurities of the water and reagents become an important factor When dealing with proteins we should also take into account the fact that they may denature at very low dilutions In order to overcome these difficulties we thought that it would be possible to obtain dispersed macromolecules either isolated or in small groups by turning the solution into an aerosol and then depositing the micro drops on the film The aerosol was obtained by means of the apparatus of Doutrebande in which filtered air was injected at a pressure of 20 lb per sq inch

Using concentrated dextrin solutions it was possible to demonstrate that the size of the micro drops generally ranges below 1 μ being in most cases of about 0 1 to 0 01 μ The aerosol obtained in these conditions is very stable and it would take very long for it to sediment under the action of gravity alone Because of this we employed electrostatic precipitation As seen in the figure once the aerosol is produced it passes through a meshwork that has been electrostatically charged by connecting it with one of the poles of a source of direct current of 2 to 3000 volts The aerosol then enters the precipitation chamber

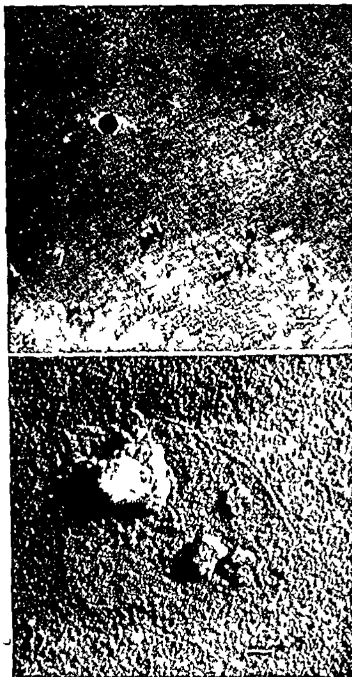


Fig 11 —Fibrinogen molecules prepared by the aerosol technique Shadow cast with palladium at 11 69 000 ×

Fig 12 —Molecules of purified horse carboxihemoglobin Aerosol technique Shadow cast with palladium at 6 8[illegible] 000 ×

containing two electrodes one of which is connected to the opposite pole of the power source The grids covered with a parlodion film are placed on this last electrode Both the meshwork and the precipitation plate are connected to the power source and the aerosol current is made to pass through The microdrops when passing through the meshwork acquire an electrostatic charge which they later lose when approaching the electrode having an opposite charge and become deposited on it Within a period ranging from 1 to 5 minutes a sufficient number of microdrops for observation with the electron microscope after shadow casting is obtained Different macromolecules were studied by means of this technique Here we shall deal preferentially with two blood proteins fibrinogen and carboxihemoglobin

Bovine fibrinogen (bovine fraction I Armour) was dissolved in buffer solution having a pH of 7 0 at a concentration of 0 06 % It was then dialyzed in cellophane tubes against distilled water at 4 C for 24 hours Crystallized horse carboxi hemoglobin possessing a high degree of purity was dissolved in bidistilled water



Fig 13 – Ultrathin section of normal human bone marrow Osmic acid fixation 7 000 ×

at pH 7 at concentration of 1×10^{-8} to 1×10^{-7} molar The molecular weight of this carboxihemoglobin is of 67 000

The results obtained for fibrinogen can be seen on Fig 11 Aside from clumps of macromolecules isolated particles can be seen The diameter of these particles is quite uniform and minimal being of about 100 A They are spherical or slightly o oidal in shape

These particles probably correspond to isolated fibrinogen molecules whose molecular weight is according to Edsall and coll (1947) and Nanninga (1946) of 440 000 We have never found elongated molecules of 600 to 700 A as claimed by Hall

Carboxihemoglobin molecules appeared in monomolecular layers or forming microcrystalline figures arranged in a more or less regular manner (Fig 12) The diameter of these molecules is of about 55 A and it is possible to distinguish them on the supporting film only on account of their distribution within the aerosol drops

Preliminary Observations in Ultrathin Sections of Bone Marrow

The considerable advances made in these last years in the technique of ultrathin sectioning make it possible to undertake the study of the ultrastructure of the hematopoietic organs both in normal and pathological conditions A wide field of research is thus opened Here we only want to present some images of human bone marrow obtained by bone aspiration (Fig 13) These preliminary observations make us foresee the possibilities opened by this technique and show us the great number of new morphological details that can be seen in the cells of the normal blood lineage

Estudio con el Microscopio Electrónico de la Sangre Circulante

El análisis con el microscopio electronico de los elementos sanguíneos circulantes ha adquirido en los ultimos anos una importancia considerable y promete convertirse en un futuro proximo en un examen de rutina para la clínica y diagnóstico de ciertas enfermedades

En este Departamento se han adaptado y desarrollado una serie de técnicas que facilitan la observacion con el microscopio electrónico de las membranas de los glóbulos rojos los leucocitos las plaquetas y las proteínas macromoleculares de la sangre

Se describirán algunas de las características submicroscopicas de los leucocitos y de las plaquetas normales extendidas sobre films de plástico

Se han efectuado estudios sobre la ultraestructura de las plaquetas en distintos estadios de su extensión sobre el film y sobre la acción plaquetolitica de las soluciones de trombina y del plasma sanguíneo Con esta técnica se puede también analizar el proceso macromolecular de la coagulación de la sangre con la presencia de todos los componentes normales que intervienen en el proceso

Serán mencionados estudios y observaciones sobre la ultraestructura normal de la membrana del eritrocito Se ha desarrollado una técnica de pseudoréplica que permite el estudio bajo el microscopio electronico de extendidos de sangre hemolizada Con ella se han hecho observaciones en eritrocitos normales de distintas especies y en animales infectados con diversas parasitosis intracelulares o en enfermedades producidas por virus

En cobayos inoculados con el virus de la aftosa aparecen masas redondeadas de alta densidad electronica formadas por pequenas partículas La presencia de estas masas coincide con la infectividad del globulo rojo

En terneros inoculados se han hecho observaciones sobre los parásitos intraglobulares *Piroplasma bigeminum* y *Anaplasma marginalis*. En la anaplasmosis se descubrió la naturaleza compleja del parásito y la presencia de cuerpos elementales de dimensiones submicroscópicas. Estas observaciones tienen importancia desde el punto de vista de la evolución clínica de la enfermedad, el ciclo vital del parásito y el tratamiento.

Se mencionarán observaciones preliminares en eritrocitos humanos normales y en diversas enfermedades incluso en leucosis agudas.

Se ha desarrollado una técnica para el estudio de macromoléculas con el microscopio electrónico. Entre otras se han logrado fotografiar moléculas aisladas de fibrinógeno y de hemoglobina. Se discutirán las posibilidades de esta técnica en el estudio de diversos fenómenos en los que las proteínas de la sangre tienen un papel preponderante.

Han colaborado en distintas fases de estos estudios los Dres. Bernardo Epstein, Pedro Lasevro, Nisio M. Fonseca, Magdalena Reissig, C. M. Franchi y Miguel Podolski.

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II 2

North American Contributions to the Histochemistry of Blood Cells

OLIVER P JONES*

HEMATOPOIETIC organs are influenced by alterations in cellular metabolism either within the marrow and lymphatic tissues themselves, or within some distant organ In many instances blood cells reflect these changes in routine preparations in others it is impossible to detect qualitative deviations beyond the normal range of variation In the latter instance it is possible with suitable technics to demonstrate that similar appearing cells may be quite different cytochemically [1] Therefore in order to unfold the functional activities and chemical constitution of blood cells it is necessary to use methods of chemical significance which will characterize lipids carbohydrates proteins enzymes and inorganic substances within their natural location [2] However it is unfortunate that some hematologists think histochemistry is a magic invocation and do not realize that it will never be any better than the accompanying morphological insight to which it is properly only an adjunct [3]

Biochemical technics have been extremely valuable for quantitating various chemical substances within tissues [4] It has remained however for histochemistry to increase the power of resolution and extend morphology so that there is an understanding of the localization of chemical constituents within cellular structure

For more than 5 years we have known something about the chemical nature of metachromasia basophilia hyaloplasm specific granulation and Auer rods [5] So let us move on to consider some of the more recent North American contributions to the histochemistry of blood cells

In the past many hematologists have no doubt noticed that chromatin in the interphase nucleus stains more lightly than chromatin in metaphase and anaphase chromosomes and have dismissed it as a result of faulty technic Orr Hardy and Pomerat [6] using Jacobson s method for staining nucleoprotein [7, 8] demon

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strated in tissue cultures that chromosomes of prophase and telophase stain reddish purple since they contain little ribonucleoprotein During metaphase and anaphase when ribonucleoprotein has been dissipated from the nucleolus chromosomes stain dark blue Jacobson and Webb[7, 8] have shown that during anaphase movement ribonucleoprotein is found in the cytoplasmic area between the two groups in a concentration higher than in any other part of the cytoplasm This nucleoprotein appears to be shed from the chromosomes into the cytoplasm Jacobson and Webb also noted that when pyronin only was used meta and anaphase chromosomes stained bright red as for ribonucleoprotein whereas prophase and telophase chromosomes were left colorless

The latter brings us to the problem of differential staining of similar acid substrates by two basic dyes Kurnick[9] has shown that methyl green stains selectively highly polymerized desoxyribonucleic acid and fails to stain to any significant extent depolymerized desoxyribonucleic acid and ribonucleic acid Pyronin stains preferentially low polymers of nucleic acid Taft[10] disagrees with this theory but considers that the question of the specificity of these stains for the demonstration of nucleic acids in tissue sections has not been completely decided

A tremendous amount of effort is being placed on studies of isolated nuclei after fragmentation of tissues and separation by differential centrifugation Since we are interested primarily in blood cells this narrows the literature considerably Allfrey Stern Mirsky and Saetren [11] in order to prevent proteins and other water soluble nuclear components from diffusing out of the nucleus and cytoplasmic and serum proteins in during isolation found varying proportions of cyclohexane and carbon tetrachloride better suited for enzyme studies than citric acid or sucrose solutions Isolated chicken and goose erythrocyte nuclei contain 19 per cent hemoglobin by dry weight Since the nucleo histone fraction is approximately 64 per cent of the erythrocyte nucleus more than half of the remaining protein is hemoglobin [12] In contrast to the fowl erythrocyte nucleus myoglobin of beef heart is entirely confined to the cytoplasm [12] To test the possibility that nuclear hemoglobin represented cytoplasmic contamination which was ground into the nuclei calf thymus nuclei were prepared from a mixed suspension of thymus tissue and avian erythrocyte debris The isolated nuclei were white in contrast to the brown coloration of erythrocyte nuclei and their normal iron content indicated that no contamination by extraneous hemoglobin had occurred [11]

If we inject some general cytology into the discussion at this point the validity of the interpretation by Stern and his colleagues may be questioned Biologists have shown that reconstruction of daughter nuclei is by the vesiculation on contiguous chromosomes and that the integrity of the chromosomal vesicles is maintained during interkinesis [13] After anaphase chromosomes absorb fluid from the cytoplasm and become vesicles As these vesicles continue to swell they unite into a variable number of karyomers and finally into a single nuclear vesicle [14] Warren Lewis[15] states that some of the evidence for this is the normal occurrence of nonadherent chromosome vesicles in invertebrates and fish the

occasional occurrence of aberrant ones in the cytoplasm of malignant fibroblasts and the amitotic fragmentation of fibroblast nuclei into two or more parts as adhesion between the vesicles diminishes in old cultures

My observations[16] with the phase microscope of primitive and definitive erythroblasts mounted in a lactic acetic-formaldehyde-gelatin medium without orcein[17] have led me to believe that after chromosomes have lost their individuality by coalescence the nuclei are no longer able to undergo mitosis When such a state is reached in orthochromatic normoblasts the nuclear membrane is no longer uniform but contains invaginations presumably between adjacent karyomeres Hence the nuclear area is invaded so-to-speak by central extensions of the cytoplasm Therefore a cytoplasm rich in hemoglobin invades the nucleus If these conditions obtain in the chicken and goose erythrocytes it is not unreasonable to expect some hemoglobin within the isolated nuclei This would tend to explain why Allfrey and Mirsky[18] could not detect differences between the nuclear and cytoplasmic hemoglobins by the N[15] uptake into hemoglobin They did however find that the rates of N[15] glycine incorporation into hemoglobin indicate that the nucleated fowl reticulocyte is more than twice as active in this respect as the fowl erythrocyte[15] Finally it seems justifiable to question the assumption by Allfrey and co workers that the condition of the nuclear membranes of calf thymus nuclei during interphase is identical with those of the mature nucleated fowl erythrocyte

In my studies it has been possible to influence embryonic erythropoiesis in the rat by administering various anti anemic substances to pregnant rats[19, 20, 21] Therefore the zinc leuco patent blue reaction for pseudo-peroxidase of Fautrez described by Lison[22] and modified by Dunn[23] might show increased nuclear activity *under these conditions* The cytoplasm *of primitive erythroblasts from* the embryonic rat yolk sac had a definite reaction[16] The patent blue reaction of these embryonic cells was not as intense as that of the mature maternal erythrocyte Although nuclei of primitive erythroblasts were essentially negative it was not possible to rule out the fact that some of the patent blue reaction within the nucleus was not due to the thin layers of cytoplasm over and beneath the nuclear area in dry smears The cytoplasmic reaction to patent blue seemed to be more intense in embryos whose mothers had received injections of pteroyl glutamic acid citrovorum factor and liver extract It was impossible to determine whether or not any of the nuclear stain had diffused into the cytoplasm but this seems unlikely since the maternal cell consistently reacted more strongly than the primitive erythroblasts

Isolated nuclei have also been investigated for their total lipid content[4, 5] Recently Wang and his co workers[6] reported that the lipoprotein complex of the nuclei of ox spleen and chicken erythrocytes contain approximately 10 per cent lipid which gives positive tests for phospholipid and cholesterol Stoneburg[1] believes that special phospholipids occur in the nucleus in relatively large amounts and that these lipids possess physical and chemical properties which point to a structural rather than a metabolic function

Although we know from chemical analyses that nuclei contain lipids the

results of direct staining methods with Sudan black and benzpyrene[7] indicate that it is the exception rather than the rule to demonstrate lipids in cell nuclei. In my preparations (first slide) of primitive erythroblasts the nuclei are tinged with a faint grey at best.[16] This has been attributed to the lipid being inaccessible to Sudan in a lipoprotein complex. Ackerman[8] has recently described a technique for producing lipophanerosis and thereby unmasking sudanophilic lipids in lipoprotein complexes. Ackerman[8] found that after suitable fixation of blood films *any one of 4 carboxylic acids (acetic 25 per cent, citric 5 per cent, formic* 10–25 per cent, and oxalic 10 per cent) applied for 2–5 minutes would unmask the nuclear lipids and make them available to staining in a 70 per cent alcoholic solution of Sudan black. The nuclei, however, in contrast to the usual blue or black coloration with Sudan black, stained a yellow brown to brown. Mineral acids such as hydrochloric, sulfuric, nitric, periodic and carbonic failed to unmask any sudanophilic material. Ackerman[8] also showed that the lipid nature of the sudanophilic material is suggested by the solubility of this component in alcohol, ether, hot pyridine and partial solubility in acetone.

We have applied Ackerman's technic to smears of a human fetal liver (C. R. 85 mm.)* in which the majority of blood cells were definitive erythroblasts.[16] The first slide† shows cells in a routine preparation (M. G. G.). The second slide is a Sudan black preparation stained for one hour at 38 C. The nuclei are practically sudanophobic and the perinuclear cytoplasmic organoids are sudanophilic. The third slide is an acetic Sudan black preparation. Here the acetic acid has not disturbed the sudanophilia of the perinuclear organoids, but it has produced a yellow brown tinge to all of the nuclei. The next slide is a citric acid Sudan black preparation. The sudanophilia of the cytoplasmic organoids has been decreased, but that of the nuclei has been increased to a darker brown. Ackerman[8] believes that the possibility of the lipid existing as a lipoprotein complex, perhaps a liponucleoprotein complex, is indicated by the solubility of the sudanophil component by various proteolytic enzymes, including desoxyribonuclease.

Hall and Deane[9] studied an acute lymphatic leukemia (AK4) in mice by means of various histochemical tests. In presenting this paper, Hall pointed out that following fixation, staining with Sudan black in 70 per cent alcohol for one hour at 38 C, there were numerous fine grey lipid granules in the periphery of the cytoplasm of lymphocytes. The granules were spherical or in the shape of short rods. They were absent from the clear rim of cytoplasm which surrounds the nucleus. Because their distribution was essentially the same as that of the phosphotungstic acid hematoxylin granules, Hall suspected that they might be phospholipid, possibly mitochondria. However, Bloom and Wislocki[20] were able to stain inclusions without the use of heat in lymphoblasts, myeloblasts and normoblasts.

Lillie and Burtner[21] have studied the sudanophilia, peroxidase and oxidase

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activities of human polymorphonuclear leukocytes Treatment of blood films with some reagents destroys or preserves sudanophilia peroxidase and oxidase activities equally with others sudanophilia is more resistant For example the glycols promptly destroyed oxidase and peroxidase and were resisted several times as long by the sudanophil substance Also when blood films are treated with 30 to 50 per cent alcohol at 60°C sudanophilia of the leukocyte granules is still demonstrable after 2 weeks but oxidase and peroxidase activities are destroyed in 30 to 60 minutes Lillie and Burtner[31] concluded that the behavior on treatment with adverse reagents and with heat indicate a similarity of benzidine peroxidase and indophenol oxidase but a dissimilarity of sudanophil substance They also found that sudanophilic granules of leukocytes are highly resistant to decolorization by absolute alcohol xylene acetone chloroform mineral oil etc for prolonged periods Restaining after 60–80 per cent alcohol decolorization is only partly successful Since the above indicated the probability of a chemical change rather than a simple physical solution of the dye in the lipid the reactive groups of the Sudans were esterified and etherified Lillie and Burtner[31] reported benzoylated and acetylated naphthol and naphthylamine Sudan dyes readily stain true fats and lipids but fail to stain leukocyte granules at 37°C

Since we have been successful in demonstrating that cytoplasmic organoids are sudanophilic in primitive and definitive erythroblasts[3, 33] it was decided to try benzoylated and acetylated Sudan black B * with the hope that if this property of sudanophilia is due to a chemical reaction the reactive groups might be blocked[16] However it was realized that acetylation and benzoylation of Sudan black might change its solubility in fat as well as in aqueous solution decrease its rate of diffusion into cells and probably decrease the protein binding of the dye[34]

The next slide shows the benzene azo naphthyl azo naphthyl structure of Sudan black B with its two reactive amine groups[35] The benzoylated and acetylated forms are also shown The second slide shows a control Sudan black preparation of definitive erythroblasts in a smear of human fetal liver (C P 85 mm) Perinuclear cytoplasmic organoids are sudanophilic The third slide is a similar preparation stained with benzoylated Sudan black and it is entirely sudanophobic The last slide of this series shows an unsuccessful attempt to unmask the lipids with acetic acid and then stain them with benzoylated Sudan black Acetylated Sudan black behaves in a similar manner All of this leads us to believe that Sudan staining of cytoplasmic organoids may not be a simple physical solution of the dye in the lipid but rather a chemical change[16] Free fat however in these smears stains with all Sudan compounds Our preliminary experiments with descending strip paper chromatography using 70 per cent alcohol as the solvent show that as Sudan black ages a yellow to yellow orange component separates out from the original drop[16]

Before leaving the subject of sudanophilia we should consider a slightly

* Furnished through the courtesy of the National Analine Division Allied Chemical and Dye Corporation Buffalo New York U S A

results of direct staining methods with Sudan black and benzpyrene[17] indicate that it is the exception rather than the rule to demonstrate lipids in cell nuclei. In my preparations (first slide) of primitive erythroblasts the nuclei are tinged with a faint grey at best.[18] This has been attributed to the lipid being inaccessible to Sudan in a lipoprotein complex. Ackerman[8] has recently described a technique for producing lipophanerosis and thereby unmasking sudanophilic lipids in lipoprotein complexes. Ackerman[8] found that after suitable fixation of blood films, any one of 4 carboxylic acids (acetic 25 per cent, citric 5 per cent, formic 10–25 per cent, and oxalic 10 per cent) applied for 2–5 minutes would unmask the nuclear lipids and make them available to staining in a 70 per cent alcoholic solution of Sudan black. The nuclei, however, in contrast to the usual blue or black coloration with Sudan black, stained a yellow brown to brown. Mineral acids such as hydrochloric, sulfuric, nitric, periodic and carbonic failed to unmask any sudanophilic material. Ackerman[9] also showed that the lipid nature of the sudanophilic material is suggested by the solubility of this component in alcohol, ether, hot pyridine and partial solubility in acetone.

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alkaline phosphatases Only traces of these reactions occurred in the intergranular cytoplasm On the basis of size and staining with periodic acid Schiff reagents two types of mast cells with intermediate forms were distinguishable One compact cells with both granules and intergranular cytoplasm stained an intense red and the other larger cells in which the reaction mainly intergranular was faint or negative Prolonged oxidation with a stronger solution of periodic acid increased the number of stained cells Since *in vitro* experiments have shown that periodic acid Schiff reagents stain heparin monosulfuric acid the variable staining of the rat s mast cells may reflect differences in their content of monosulfated heparin The staining of increased numbers of mast cells after more prolonged oxidation may be attributable to the partial conversion of unstained heparin to the stainable monosulfated form Wislocki and Fawcett[4] believe that the vivid metachromasia in the mast cell granules would seem to be a more reliable indication of the cytological location of the reaction than the evidence obtained from centrifugal fractionation of the cells

Wislocki Rheingold and Dempsey[40] also found that tissue and blood eosinophil granules are not influenced by salivary digestion The cytoplasmic ground substance in the eosinophils of human blood is chiefly colored About 10 per cent of human lymphocytes contain a few deep red periodic acid-Schiff positive granules which resist amylase These cells are increased both relatively and absolutely in blood from patients with chronic lymphatic leukemia In fact Wislocki, Rheingold and Dempsey[40] reported one case in which practically all of the lymphocytes contained from 6 to 12 bright red dots The latter is not in agreement with Wachstein s observations in lymphatic leukemia [43]

The nature of staining mechanism has been recently reviewed by Singer[44] and experiments have been conducted by Weiss[45, 46] to obtain further information regarding the electrochemical composition of blood Dye uptake by tissue structures is influenced by fixation the nature of the dye and its concentration the temperature and duration of staining and pH [46] Weiss used reagents to selectively block or destroy certain chemical groups in blood cells

Preliminary studies suggest that histochemical technics may prove to be valuable in the differentiation of the lymphoblastomas Ackerman Knouff and Hoster[47] studied biopsy specimens of 102 lymph nodes from a wide variety of disorders and evaluated them according to their ribonucleoprotein content (basophilia) periodic acid-Schiff reaction sudanophilia and alkaline and acid phosphatase activity Modified lymphocytes of lymphosarcoma chronic lymphatic leukemia and infectious mononucleosis contain more glycogen and mucopolysaccharide granules than do normal lymphocytes There is cytochemical and morphological evidence for the formation of Hodgkin s cells from normal elongated reticulum cells The earliest changes occurring during this transition are a gradual increase in acid phosphatase of the nucleus and nucleolus followed by an increase in the ribonucleoprotein of both the cytoplasm and nucleolus and elaboration of lipid and mucopolysaccharide granules Following alkaline and acid phosphatase reactions leukemic lymphocytes usually have one large nucleolus while the sarcomatous lymphocytes contain several

different use of these dyes in studying cellular activity Wooton Ellinger and Bartone[36] studied the phagocytic activity in the peritoneal cavity of white rats after they had been injected with cod liver oil previously stained with Sudan IV Histiocytes were more active than the fibrocyte like cells in absorbing fat Ingestion by the cells of the previously stained fat took place at specific points within the extensive interface between cell and fat and the ingested fat was observed as streaks within the cytosome The presence of lipase at the cell fat interface which was identified by the Gomori method suggests the possibility of an extracellular digestion of fat as part of the absorption phenomenon

Auer rods have been studied by Ackerman[37] in cases of acute monocytic and myelocytic leukemia According to Ackerman[37] they are oxidase peroxidase and periodic acid Schiff positive sudanophilic slightly metachromatic, and give positive tests for acetal lipids and ribonucleic acid He believes that some alteration must exist in leukemic cells which permits newly forming granules to coalesce into crystal like rods The coacervate nature of Auer rods was suggested by their presence almost always within young cells whose granules were of a relatively acid pH the formation of vacuoles and globules with increased time and temperature and the finding of nongranular areas about the developing Auer bodies

The Schollenkenkozyten or globule leukocytes have been investigated extensively in the rat urinary tract by Kirkman[38] and in the alimentary tract of sheep by Kent [39] Kirkman lists 80 *differential characteristics of these cells* and of certain possibly related forms He concludes that they are of connective tissue origin but their relationship to plasma cells with acidophilic inclusions and Russell body cells remains uncertain Kent[39] found that the globule leukocytes of sheep contain iron but do not give the hemoglobin reaction phosphatase but not stable oxidase and labile peroxidase The biuret reaction for the peptide linkage was weakly positive and the reaction for arginine positive and they are sudanophobic However the cytoplasm of these cells did not contain small sudanophilic particles presumably mitochondria or Golgi material These cells are distinct from eosinophilic leukocytes Russell body cells and erythrophagocytic cells

The Bauer Feulgen technic according to Wislocki Rheingold and Dempsey [40] is apparently not as sensitive as the periodic acid Schiff reaction for a wide variety of acid mucopolysaccharides It is likely that the substances detected are (a) glycogen which may be removed from sections by digestion with saliva (b) mucoproteins and (c) chiefly mucopolysaccharides presumably mucoitin sulfuric acid heparin and hyaluronic acid [41] Wislocki Rheingold and Dempsey[40] found that certain periodic acid Schiff positive cells resist amylase digestion and therefore contain a carbohydrate other than glycogen This reaction indicates that there may be a species difference in tissue mast cells and there may be a difference between the tissue mast cell and basophil leukocyte

Last year Wislocki and Fawcett[4] reported that mast cell granules in the normal rat mesentery consist of an intensely metachromatic central core coated by a material which is sudanophilic and reacts positively for acid and

la fórmula química del Sudán negro es actualmente conocida, hay todavía muchos interrogantes con respecto a las propiedades sudanófilas. La naturaleza del solvente, edad de la solución, tipo de fijación, duración de la coloración y la temperatura, todo influencia estas propiedades.

La coloración de los lípidos por el Sudán puede no ser simplemente una disolución física del colorante en los lípidos, sino más bien un cambio químico.

Todos los tipos de granulaciones han sido estudiadas con varias reacciones. Cuando los neutrófilos son tratados con alcohol al 30-50 % a 60°, la sudanofilia es todavía demostrable después de dos semanas de exposición, pero la actividad oxidásica y peroxidásica es destruída en 30 a 60 minutos. No hay similitud de la substancia sudanofilia con bencidino peroxidasa e indofenol oxidasa.

Los cuerpos de Auer son oxidasa, peroxidasa, reacción ácida de Schiff periódica positiva, sudanofílicos, ligeramente metacromáticos y dan positiva la prueba para lípidos acetales y ácido ribonucleico.

Los glóbulos de la Schollenleucozyten o glóbulos leucocíticos contienen hierro, pero no dan la reacción de la hemoglobina, fosfatasa, pero inestable oxidasa y lábil peroxidasa. La reacción del biuret para las uniones péptidas es débilmente positiva y la reacción para la arginina positiva y son sudanófobos.

La técnica de Bauer Feulgen es aparentemente no tan sensitiva como el ácido Schiff periódico para una amplia variedad de mucopolisacáridos ácidos. Ciertas células ácido Schiff periódico positivas resisten a la digestión de la amilasa y por lo tanto contienen un carbohidrato distinto del glucógeno. Esta reacción indica que hay una especie diferente en los mastzellen de los tejidos y que puede haber una diferencia entre los mastzellen de los tejidos y los leucocitos basófilos. Los gránulos eosinófilos de la sangre y de los tejidos resisten la digestion salivar. Alrededor de un 10% de los linfocitos humanos contienen unos pocos gránulos rojo intenso ácido Schiff periodico positivo y resistentes a la amilasa.

Estudios preliminares hechos en biopsias de ganglios linfáticos humanos demuestran que las técnicas histoquímicas pueden ser valiosas en la diferenciación de linfoblastomas porque alteraciones citoquímicas tienen lugar antes de que puedan apreciarse cambios morfológicos. Los linfocitos modificados del linfosarcoma, leucemia linfática y mononucleosis infecciosa contienen más glucógeno y gránulos mucopolisacáridos que los linfocitos normales. En las células iniciales del Hodgkin hay un aumento gradual en la fosfatasa ácida de los nucleos y nucléolos seguido por un aumento de las ribonucleoproteínas del citoplasma y de los nucléolos y una elaboración de lípidos y gránulos mucopolisacáridos. Después de las reacciones de fosfatasas ácidas y alcalinas los linfocitos leucémicos muestran generalmente nucléolos grandes y los linfocitos sarcomatosos varios y pequeños nucléolos.

La metacromasia en las células y tejidos ha sido asociada con mucopolisacáridos, nucleoproteínas y substancias de composición desconocidas. Dos tipos de mastzellen naturales con formas intermedias han sido observados.

Las mitocondrias en los leucocitos parecen colorearse más profundamente con la hematina ácida de Baker que con el Sudán negro sugiriendo que ellas son ricas en fosfolípidos. Han sido estudiadas en frotis secos de sangre de embrión por medio de la técnica del azul de anilinafucsina básica modificada.

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small nucleoli Cytochemical *alterations may take place before morphologic* changes are discernible

It is with this last thought in mind that I want to bring this paper to a close We know that cytoplasmic organoids—chiefly mitochondria—are preserved in good dry smears[37] We also know that cytoplasmic organoids on either side of flattened nuclei contribute to the formation of the nuclear pattern of immature blood cells[33] The importance of mitochondria and their relation to enzyme systems in the processes of cellular respiration have been emphasized by Harman and others[48, 49, 50] Alterations in the staining reaction during mitosis of tumor cells have been reported by Dalton[51]

Some years ago it occurred to me that in hematology we needed a simple rapid and permanent mitochondrial technic that avoided the use of heat In order to accomplish this in a preliminary manner it was necessary to borrow from the endocrinologists and adapt the basic fuchsin aniline blue technic described by *Fain and Wolfe*[5] *to dry blood films My technic is applicable only* to embryonic blood cells because the vagaries of this technic when applied to bone marrow have not as yet been mastered[53, 54] The results show promise and this line of attack should be pursued in order to correlate changes in mito chondrial structure with function especially in very immature leukemic cells

The first slide is a routine preparation of blood cells from the embryonic rat yolk sac These cells are all primitive erythroblasts The second and third slides show mitochondria during interphase The fourth slide illustrates mito chondria grouped between two telophase nuclei The fifth and sixth slides demonstrate the appearance of a constriction band dividing the mitochondria into two more or less equal groups In the seventh slide the mitochondria are arranged in parallel groups adjacent to the equatorial plate In the eighth slide mitochondria are in two groups Although daughter nuclei have been recon structed cytokinesis has not occurred The last slide shows asymmetrical mitosis a relatively common finding which undoubtedly contributes to the anisocytosis prevalent in embryonic blood

In conclusion let me say that it is hoped some of these investigations will lead to a better understanding of blood cells and their function with the goal of making earlier and better diagnoses of hematopoietic disorders and the proper treatment of them

Contribución Norteamericana a la Histoquímica de las Células Sanguíneas

La tendencia en histoquímica ha sido primeramente subordinar un grupo de tejidos a un simple procedimiento técnico como una medida exploratoria Después un simple tipo de células han sido objeto de una batería de pruebas histoquímicas para finalmente someter a cada uno de los constituyentes celulares a los mismos

Las reacciones de coloración de los nucleos han sido estudiadas por medio de las nucleasas para *saber por qué* los nucleos y los cromosomas reaccionan de ese modo a la pironina verde de metilo y al May Grunwald Giemsa Nucleos aislados de eritrocitos de pollo y ganso contienen hemoglobina Agregado a ésto hay complejos lipoproteicos en los núcleos del bazo de buey y eritrocitos de pollo que contienen aproximadamente el 10% de lípidos

Lípidos en los núcleos de los frotis sanguíneos de rutina *han sido descubiertos con los* ácidos cuatro carboxílicos y posteriormente se han demostrado con el Sudán negro Aunque

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II 3

Microspectrographic Methods in Hematology (with an Example of Leukemic Cell Analysis)

BO THORELL*

THE RAPID development of histo- and cytochemistry in the last 15 years has made possible the accumulation of data on the chemical composition and cytologically defined structure of cells. Through the study of cells under normal and pathological conditions it may be possible to relate the manifold physiological functions and properties of a cell to its structural pattern.

There are at present two main groups of quantitative cytochemical methods. The first includes methods in which chemical analysis is made on isolated cytologically defined parts of a cell population. Such an isolation and purification is often obtained by differential centrifugation or flotation techniques. The chemical data yielded by these methods are fairly good. The preparation of the sample, however, may introduce errors such as uncontrollable extractions and the procedure must be critically examined in each case. In the second group of methods the definition and localization in the cell is obtained with the measuring technique itself. This group mainly consists of methods using absorption measurement with radiant energy such as microspectrography in the infrared, visible, ultraviolet and X-ray spectral ranges. These procedures are technically somewhat complicated.[1-4]

It has been evident that microspectrography is a very sensitive method of analysis[1] in that very small amounts of substance can be determined when they occur in relatively high concentrations. Substances in high dilution cannot be analyzed. Also the precision by which a microspectrographic analysis can be performed is not very high compared to the usual standards in physical chemistry. Generally the error is considerably higher than 10%. The limit of accuracy varies from one type of cell to another depending upon different properties such as the distribution and heterogeneity of the cellular structures containing the absorbing material.

But as in most cases in biological research as well as cytochemistry one single method cannot give the answer to a problem. The microspectrographical technique has its greatest value when it is combined with other methods.

The microspectrographic analysis of the normal development of the bone marrow cells[4] has shown a characteristic decrease in the concentration of cytoplasmic nucleic acids during maturation. We have interpreted this as a sign of a continuously decreasing metabolic activity of the protein synthetic processes during blood cell maturation.

The microspectrographic analyses of leukemic cells show that these cells

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The experiments in collaboration with Dr. A. Fagraeus on leukemia cells have been kindly supported by a grant from the Lady Tata Memorial Trust.

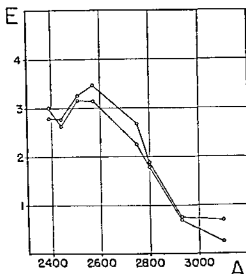


FIG 1—The ultraviolet absorption from points in the cytoplasm of acute leukemia cells The curves show a maximum around the wavelength of 2600 A The extinction values of these cytoplasms correspond to a ribonucleic acid concentration of 5–7% i e on an average higher than in the normal hemocytoblast For details see ref 4

accumulate great amounts of nucleic acids in their cytoplasm[4] (see Fig 1) In the virus induced leukemias one must consider both the growth processes of the cell and the multiplication of the leukemic agent The question is then To which of these processes are the cytoplasmic nucleic acids related?

To answer this question one must demonstrate the location of the virus in the cell With spectrographic methods one cannot determine any virus activity So we tried to separate the cellular constituents

Figure 2 gives a summary of the preparation procedure Table 1 shows activity titrations of the different cellular fractions It is evident that the leukemia inducing activity of the cytoplasmic fraction is approximately the same as of the total disintegrated cells calculated per unit nitrogen (The details of the e experiments will be published) At least a great part of the virus thus is structurally related to the microspectrographically demonstrable cytoplasmic nucleic acids

SUMMARY

The microspectrographic technique has a definite value when combined with other methods because it offers a method of quantitative analysis of biochemically active compounds distributed in a given cell This approach with other methods on the same material can be used to bring the findings to a point where they may be used to interpret events on the cellular level

METODOS MICROESPECTROGRAFICOS EN HEMATOLOGIA

La técnica microespectrográfica tiene un valor definido cuando se la combina con otros métodos porque ofrece una posibilidad de análisis cuantitativo de compuestos bioquímicamente activos distribuídos en una célula dada

Esta forma de encarar el problema junto con otros métodos sobre el mismo material puede ser usado para correlacionar los hallazgos con la interpretacion de hechos en la estructura celular

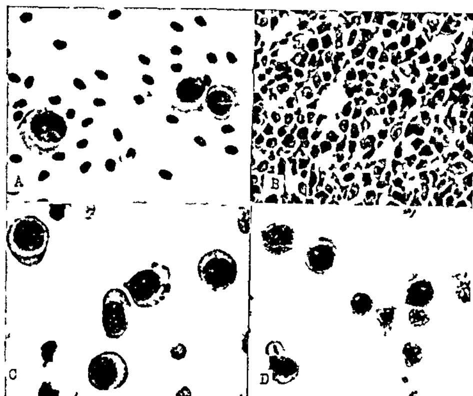


FIG 2A —Leukemia cells in a stained blood smear (erythroblastic virus strain)

FIG 2B —Stained section of the enlarged spleen from which the leukemic cells are isolated

FIG 2C —Washed cells from the spleen

FIG 2D —Stained smear of leukemic cells after treatment with a homogenizer Most of the cytoplasms have been scratched off without destroying the nuclei This procedure must in each experiment be rigorously controlled by phase contrast microscopy and counting of nuclei before and after treatment The nuclei are centrifuged down and the fairly clear supernatant is regarded as cytoplasm

TABLE 1 *Titration values of the leukemia inducing activities of total disintegrated cells cytoplasmic extract and nucl i The titrations were made using an inbred strain of Leghorn chickens Spontaneous leukemia in this strain less than 0 1% Number of injected chickens within brackets The table shows number of chickens dead in histologically verified leukemia within 3 weeks The 50% endpoint titer is calculated according to Reed and Muench Am J Hyg 27 493 1938*

D l t	C lls	Cyt pl m	N l i
10^{0}	—	—	3(5)
10^{-}	—	4(5)	2(6)
10^{-2}	5(6)	1(5)	?(5)
$10^{\ 3}$	1(5)	0(5)	3(6)
10	0(5)	0(5)	0(5)
$10^{\ 5}$	0(5)	0(5)	—
50% endpoint titer	$10^{-\ 12}$	$10^{\ 1\ 50}$	$10^{-\ 22}$
Total nitrogen mg/ml	0 440	0 0535	0 116
Activity/nitrogen k	1 2	1 0	0 1

REFERENCES

[1] CASPERSSON T Cell Growth and Cell Function New York 1950
[2] ENGSTRÖM A Acta Radiol Suppl 63 1946
[3] FRAZER R D B Faraday Soc Discussion 1951
[4] THORELL B Acta Med Scand Suppl 200 1947

II 4

General Considerations of the Structure and Physiology of the Nuclear Apparatus

I GONZALEZ GUZMAN*

IT IS intended to show in this report by means of diagrams the structure of the nucleolar apparatus It is much more complicated when the nucleoli are studied through procedures of selective stains Also considered are the functions of this little cellular organ

Structure of the nucleolar apparatus

I Morphology and structure of the nucleolar apparatus
- (a) The nucleolus in itself
  - Membranes
    - Nonapparent
    - Apparent chromatinic Lipoproteic
  - Fundamental substance
  - Lipoproteic materials granules cortex complete or incomplete
  - Nucleolonema
  - Vacuoles
  - Other granulations
- (b) Extranucleolar lipoproteic granules
- (c) Nucleonephelius and vacuolar system

In the next chart there are shown some data about the nucleolar membrane, beginning with the first descriptions and following with those more clearly evident

II The nucleolar membranes

Will 1885 Describe for the first time the nucleolar membrane in the ovular nucleoli Can be demonstrated with ordinary techniques

Cavara 1898 Nucleoli of vegetable cells Chromatinic fenestrate membranes

Montgomery 1899 Cells of different types It is a condensation of the fundamental substance

Saguchi 1923 Culture of conjunctive cells Basophil cortex of fuzzy inner limits

Naegeli 1923 Human lymphatic cells Cortex that dyes with methylene blue (Probably it is the fundamental substance)

Laboratorio de Estudios Médicos y Biológicos Escuela de Medicina Mexico D F



Fig 1 — A scheme of the nucleolar apparatus On the left Saguchi s scheme On the right Author s scheme

González Guzmán 1935 (a) Human lymphatic cells stained with methylene blue (b) Lymphatic cells in supravital technique (c) Double silver staining Histiocytes hemo cytoblasts Lymphatic cells Lipoproteic cortex

In the next scheme there are pointed out the accepted data about the micro scopical appearance and chemical composition of the nucleolar corpuscles

III The fundamental substance

Aspects

(a) With ordinary techniques Acidophil basophil amfophil Some times meta chromasia

(b) Mitochondrial techniques Coloration similar to that of the fundamental sub stance of chondriosomes

(c) Silverstains Rio Hortega Achucarro Like ribosa nucleic acids or lipoproteins

(d) With ultraviolet rays Great absortion at wave lengths of 2 600 Å

(e) Phase contrast Different tones of gray ranging from gray to black

Chemical composition

It contains iron Prussian blue test Scharoff

They contain glutathione Jovet Lavergne

Ribosa nucleic acid Casperson Brachet

Desoxyribosa nucleic acid Pfitzner it is formed by prochromatin Saguchi the cortex stains like chromatin Hurel and Hurel in many vegetable cells there are grains Feulgen positive into the nucleoli and filaments that unite them to chro matin González Guzmán the nucleoli of old cells have granules or chromatinic cortex

Proteins rich in exonic bases Casperson

Lipoproteins Many authors Selective staining with mitochondrial or golgian techniques

The most important data about the nucleolar lipoproteins are summarized in the next scheme

IV Lipoproteic materials

Diffuse

Blackening with osmium tetraoxide

Selective staining with mitochondrial techniques

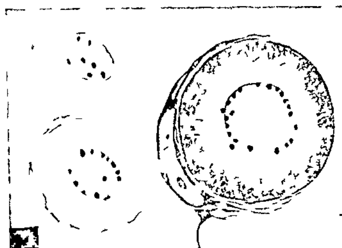


FIG 2—Evolutive scheme of the frog ovocytes during the vitelogenesis Submembranous disposition of the numerous nucleoli and the passing of some of them into the cytoplasm

Granular

Simarro 1900 Nucleoli of neurons Staining with photographic silver salts Ruby red granules

Cajal 1903 The same granules with his silver staining method

Tello 1911 Nucleoli of living cells Neurons and other somatic cells Ultraviolet rays lack of absorption

Rio Hortega 1923 Staining with double silver stain

González Guzmán 1935 1938 1942 1946 1950 1951 1952 Granular structure is universal Description in several types of cells In those of blood and hemopoietic organs Description of the nucleolar lipoidogenic cycle

The intranucleolar vacuoli have been broadly studied since the middle of the past century and interpreted differently by several authors The most important data are summarized in the next scheme

V *Nucleolar vacuoli*

Kolliker 1840 Nucleoli may have vacuoli

Mauthner 1860 They are described as nucleolar spots

Balbiani 1864 In the nucleoli of living cells movable vacuoli that empty into the karyolymph and form again

v Bambeke In ovular nucleoli A central vacuole that contains an interior granule of high motility

Lache 1905 Nucleoli of neurons Big vacuoli surrounded by fine granules stained with thionin

Muhlmann 1911 Names them lipoidosomes

Saguchi 1923 Conjunctive cells in culture One to three vacuoli usually central in their location They would be of lipoidic nature

González Guzmán 1951 1952 Phase contrast microscopy Fine vacuoli in nucleoli of hemocytoblasts myeloblasts lymphoblasts and histiocytes

The granular corpuscles contained in the nucleoli have been the subject of several descriptions and violent arguments The outstanding features are mentioned in the following scheme

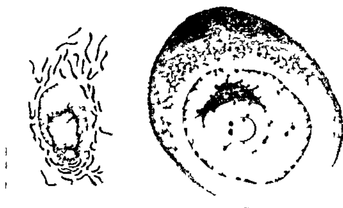


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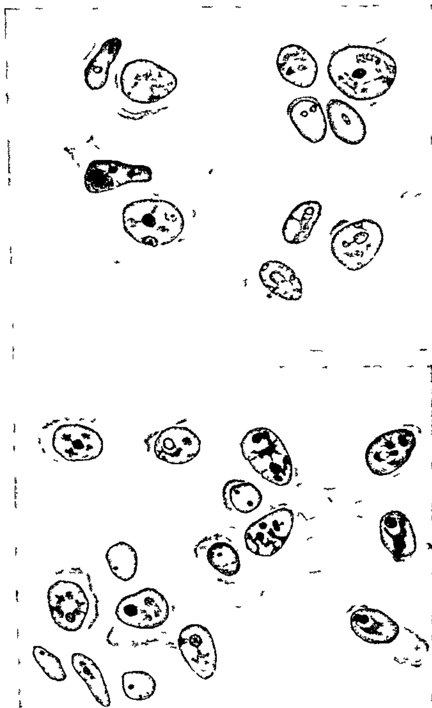


FIG 4 —Frog spleen histiocytes during the peak of the antibody formation Proteic masses recently elaborated in relation to the nucleolar apparatus



FIG 3 —Pancreatic cells showing the nucleolar apparatus the clear perinucleolar area and the proteic masses in relation to the nucleoli

I I *Nucleolar granulations*

Schron 1865 In nucleoli of young ovocytes One or two granulations stainable by anilines inside a pseudo vacuole

Eimer 1871 1872 Concentric granules right under the membrane are described

Marinesco 1898 Neurons of dog with strichnine poisoning Coarse granules stainable with aniline dyes that can go into the karyolymph

Lentz 1909 Ganglionic cells in animals with rabies Great nucleolar hypertrophy and acidophil or basophil intranucleolar corpuscles (Lentz corpuscles)

Simarro 1900 Neuronal nucleoli Argentophilic granules

Nucleoli

Agazis 1857 Description of endonucleolar corpuscle the nucleolus

Mann 1892 Points out an endonucleolus with radial filaments

The granules and corpuscles which are inside the nucleoli may come out into the karyolymph This fact has been pointed out by many investigators and the author has studied the phenomenon in relation to the lipoprotein synthesis Some of the most important data are listed in the following scheme

I II *Nucleolar granules shifting to the karyolymph*

Marinesco 1898 Dogs poisoned with strichnine Granules stained with anilines

Collin 1908 Neuronal nucleoli of the guinea pig Migration to the karyolymph

Shifting of argentophilic granules

González Guzmán 1942 to 1952 In carcinomatous cells no constant shifting but very numerous In blood stream cells frequent migration but less numerous In plasma cells constant shifting of one to three granules

The nucleolar apparatus is not exclusively constituted by the nucleoli but also by extranucleolar structures The most important ones are the juxtanucleolar vacuoles which in times are grouped perinucleolarily and the formation

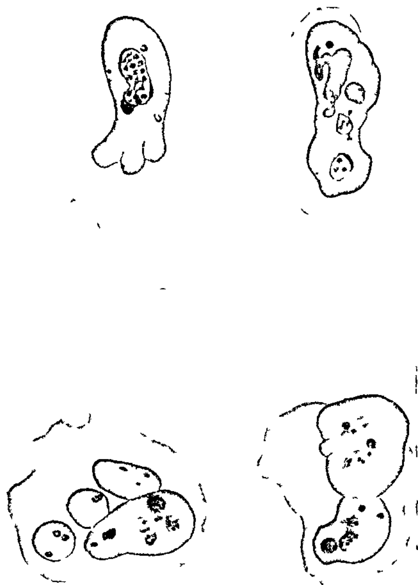


FIG 6 — Different aspects of the Sternberg cells Huge nucleolar apparatus with abnormal structures

again Occasional phenomena? Form of degeneration What substance do they contain?

Haecker 1883 The nucleolus is a pulsable organ pulsierendes organulum By confluence a vacuole is formed and then empties into the karyolymph

Nucleolar movement

Brandt 1874 They have slow amoeba like movements

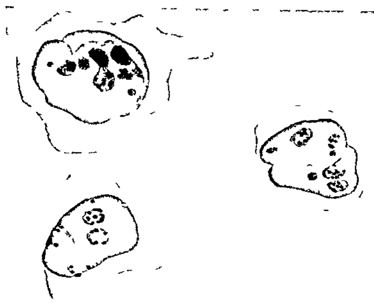


FIG 5 —Mobilized histiocytes in an early stage of the transformation to Sternberg cells

known after Saguchi nucleonephelius In the following list are enumerated some of these data

VIII *The nucleonephelius*

Saguchi 1923 Cloudy appearance without definite form of nucleolar origin
González Guzmán 1935 1950 A vacuolar system and a cloudy perinucleolar zone both formed by materials elaborated by the nucleoli

Morphology

Saguchi Cloudy and amorphus sometimes well limited with thorns Forming a crescent or a perinucleolar ring separated from the nucleolus by a clear area
González Guzmán Clear amorphus perinucleolar area or a system of vacuoli resembling a rose single or several common for two nucleoli The nucleonephelius of Saguchi would be a phase of advanced proteogenesis

Functions

Saguchi Frequent shifting of the nucleus and entering the protoplasm in the zone of cellular center It would contribute to the formation of the centronephelius When the cell is going to divide it breaks up into fragments until it disappears
González Guzmán It is the zone of intermedium proteic metabolism Clear and voluminous in the blasts or pancreatic cells disappears with maturation and differentiation

The understanding of the nucleolar functions has changed from time to time At the beginning very little was known and frequently it was erroneous but some of the suspected theories have been at the present time thoroughly developed It was with the use of new research tools that we began to know about the nucleolar functions In the following scheme we revise the old concepts and summarize the recent ideas

IX *Outline of nucleolar function*

Function of nucleolar vacuoli

Balbiani 1864 1865 Movable vacuoli that empty into the karyolymph and form

(d) Great development of the nucleonephelius and by products of intermediate metabolism

Maturation and aging cause

(a) A diminished nucleolar richness and simplification of the granular structures

(b) Progressive slackening of the protein synthesis ribose nucleic acid and lipoproteins

(c) Accumulation in the nucleolus of the prechromatin nucleotides

(d) Vanishing of the nucleonephelius

The cells with great secretory capacity have a nucleolar apparatus similar to that of young cells with great reproductive capacity

Finally in some pathological states, the structure and functions of the nucleolar apparatus are deeply modified In the next chart the most important of these changes are outlined

VII *Physiopathology of the nucleolar apparatus*

(a) *Hyperproduction of immunity globulins*

Place Reticulo endothelial system cells

Mechanism nucleolar hypertrophy with hyperproduction of ribose nucleic acid and nucleoproteins Liberation of antibodies

(b) *Hyperproduction of pathological globulins*

Example multiple myeloma

Place Reticulo-endothelial system cells chiefly of bone marrow system

Mechanism nucleolar hypertrophy with hyperproduction of ribose nucleic acid and nucleoproteins Liberation of atypical globulins

(c) *Unbalance between proteosynthesis and production of lipoproteins*

Example Hodgkin s disease

Place Sternberg s cells

Mechanism tremendous hypertrophy of the fundamental substance of the nucleolus with relative diminution of argentophilic granules Great production of ribose nucleic acid Comparative diminution of the production of lipoproteins

The ideas and problems expressed in the charts are too schematic and actually require a greater elaboration of detail than space allows here if they are to be made fully comprehensible

Estructura y Funciones del Aparato Nucleolar

El aparato nucleolar es un organito celular muy complejo en su estructura y en sus funciones Consta de los nucleolos y de un sistema de vacuolas que lo circundan el nucleonefelio El nucléolo está constituído por una membrana substancia fundamental y un conjunto de granulaciones argentófilas La substancia fundamental es el sitio de la síntesis de proteínas ricas en bases exónicas de ácido ribosa nucleico y de pequenas cantidades de ácido desoxiribosa nucleico Las granulaciones argentófilas son los organoides nucleolares encargados de la síntesis de las lipoproteínas y lipoides celulares El nucleonefelio es un sistema vacuolar inconstante desprovisto de funciones formativas simple reservorio de algunos productos intermedios del metabolismo nucleolar

Las perversiones de las funciones nucleolares conducen a estados patológicos de la célula susceptibles por su extensión y cuantía de originar diversos síndromes patológicos La evolución ontogénica del aparato nucleolar modifica su complejidad estructural y sus funciones con la madurez y envejecimiento disminuye el volumen nucleolar se hacen menos numerosas las granulaciones argentófilas desaparece el nucleonefelio y disminuye mucho la síntesis protéica y la formación de lipoproteínas

Scharoff 1896 The nucleoli that contain iron can go out into the protoplasm
Fick 1899 Bajewska 1919 Describe the nucleolar migration to the protoplasm
González Guzmán Several examples of nucleolar migration are observed

Fragmentation of the nucleoli

González Guzmán 1935 In living lymphoblast of the frog 1942 In carcinomatous cells fixed and stained

The nucleoli perform the synthesis

Of ribosa nucleic acid

Ogata 1883 The nucleolus form the Nebenkern of pancreatic cells
Holmgren 1899 From the nucleus and nucleolus of neurons come small granules that pass into the cellular center and later form Nissl corpuscles
González Guzmán Interrelation of nucleolar variation and the pancreatic secretion

Of desoxyribo-nucleic acid

Pfitzner 1883 Rabl 1885 Schultze 1887 Korschelt 1895 Auerbach 1896 Hertwig 1898 Czermak 1899 The nucleolus participates in the formation of chromatin
Saguchi 1923 The cortex zone of the nucleolus forms chromatin that breaks up into granules that are distributed in the linin net
Hurel and Hurel 1936 The nucleoli of vegetable cells have Feulgen positive granules
González Guzmán 1952 The nucleolus forms the materials (nucleotides) necessary for the synthesis of desoxyribose nucleic acids

Of the vitellus

Macallum 1891 From the nucleoli emerge fluid substances that pass to the nucleus afterwards to the protoplasm and there they contribute to the formation of the vitellus
Carnoy and Lebrun 1897 The nucleoli form paranucleic acid that passes into the nucleus and to the protoplasm where it fuses with globulins to form the vitellus
González Guzmán Studies the role of the nucleolus in the formation of the vitellus of the ovocyte of the frog

V *Role of the nucleolus in the proteic synthesis*

Casperson scheme

The associated chromatin controls the synthesis of ribose nucleotides It accumulates in the nucleolus and later passes into the nucleus and protoplasm Proteic synthesis takes place in the protoplasm in presence of large amounts of ribose nucleic acid with the aid of certain enzymes

Author s scheme

The nucleolus is not an organ of accumulation but it is a center of synthesis it forms mononucleotides poliribose nucleotides and perhaps the materials for the synthesis of the associated chromatin The nucleotides leave the nucleolus and later accumulate in the protoplasm

The complex nucleoproteins with ribose nucleic acid break it up into globulins or histones and sulfo phosphoralid catabolites The globulins or histones are specific of the cells or model their structure (antibodies) on patterns that are present in the protoplasm (antigens)

The nucleoli as the cells in which they are contained and as the organism itself have an ontogenic evolution during which they modify their functional and structural characteristics as shown in the next scheme

VI *Ontogenic evolution of the nucleolar apparatus*

Young cells of great reproductive capacity are distinguished by

(a) A voluminous nucleolar apparatus rich in argentophilic granulations
(b) For a great nucleolar production of proteins rich in exonic bases ribosenucleic acid and lipoproteins
(c) Scanty production of prechromatin nucleotides

ogists have been unable to differentiate muscle columns and transverse bands undergoing the change from erythrocyte bearing capillaries or massive release from hemorrhages

The working hypothesis based on these observations is as follows

Blood formation is the result of histolysis just as in the embryonic blood islands Normal and continuous replacement histolysis is the source of new capillaries and their cellular contents *The same process disorganized by* trauma or disease leads to extravasations inflammatory changes *deposition* of abnormal tissue components The cellular waste (more abundant if the process is pathologic) is *deposited in* the lymphoid and myeloid tissue These are regarded not as the sites of formation but as graveyards of the cellular waste and at the same time sources of raw materials

In this concept therefore hemopoiesis is intravascular in location histolytic in nature and cytomorphic in its results

Una Teoria Citomorfica de la Formacion de la Sangre

Mientras se reconoce generalmente la formación de leucocitos fuera de la médula roja se cree que la eritropoyesis—aparte de la formacion de sangre embrionaria—*está limitada* a la médula ósea Se cree que los glóbulos rojos derivan de precursores nucleados los que pierden sus nucleos en forma que varía según las diferentes teorías (desaparicion cariorrexis y cariólisis expulsión gemación) y que pasan de una manera aun inexplicada desde los sitios extravasculares de produccion a los vasos Pese a la alta tasa de producción de glóbulos rojos estos proceos no se pueden evidentemente demostrar con facilidad ya que las opiniones difieren ampliamente

Estas teorías son incompatibles con los procesos de formación de sangre y vasos como se observan en los islotes sanguíneos embrionarios donde el proceso esencial es intracelular rápido y se asemeja a la *histólisis* y no a una lenta diferenciación a través de muchas generaciones celulares

También debemos recordar

(1) que los vertebrados inferiores hasta los Urodelos no tienen médula ósea y que después de la esplenectomía (siendo el bazo considerado como el órgano hemopoyético) estas formas continuan produciendo sangre[7]

(2) que los cultivos de tejidos de la médula son muy desalentadores porque las células de la médula no continuan formando granulocitos y globulos rojos [8] después de 5 días de cultivo las células se vuelven difíciles de colorear y luego de 8 días es imposible obtener preparaciones apropiadas para su estudio[8]

(3) que la médula ósea es el unico tejido donde siempre se encuentran los cambios celulares necrobioticos (pérdida del turgor nuclear cariolisis picnosis cariomegalia y formación de células gigantes) y que por esta razón Hueck la considera juntamente con los tejidos linfoides como órgano de inflamación fisiológica

(4) que las ascendencias de las células dadas por varias teorías son arbitrarias difieren ampliamente y carecen de pruebas (Stohr)

(5) que la médula ósea frecuentemente se comporta paradojicamente haciéndose hiperplástica en las anemias graves y aplástica en las leucemias

(6) que como el tejido linfoide y especialmente el timo involuciona proceso que comienza en la pubertad

(7) que la transformación mieloide ha sido registrada *en* muchas oportunidades (recientemente en la corteza adrenal[9]) y que esta transformación va acompanada *por* cambios degenerativos

Nunca se insistirá lo suficiente en que lo que más se necesita en la evaluación de las investigaciones en este campo es más respeto por los hechos de observación por discordantes que sean con las teorías corrientes y menos respeto por las teorías por ampliamente aceptadas que parezcan

El objeto del trabajo del autor se limita al tejido muscular en actividad estudiado en estado vivo y en material *histológico* más *precisamente* los fenómenos histolíticos de la substancia *contráctil* en *tejido* intacto y *traumatizado* Sabemos desde hace más de un

II communication 1

A Cytomorphic Theory of Blood Formation

STANISLAW H WAJDA*

While leukocyte formation outside the red marrow is generally recognized it is believed that erythropoiesis—apart from embryonic blood formation—is restricted to the bone marrow Red cells are thought to be derived from nucleated precursors which lose their nuclei *in a way which varies according to different theories* (disappearance karyorhexis karyolysis extrusion gemmation) and to pass in a yet unexplained manner from extravascular production sites into the vessels In spite of the very high production rate of red cells these *processes are obviously not readily demonstrated* and opinions differ widely

These theories are incompatible with the vasoformative and blood forming processes as seen in the blood islands of living embryos [1]† where the essential process is intracellular rapid and resembles histolysis *and not a slow differentiation through many cell generations*

It should also be remembered

(1) that lower vertebrates up to Urodeles have no bone marrow and that after splenectomy (the spleen being regarded as the hemopoietic organ) *these forms* continue to produce blood[2, 3, 4]

(2) that tissue cultures of the marrow are very disappointing for the cells of the marrow do not continue to form granulocytes and red blood cells [5] after 5 days of culture cells become difficult to stain and after 8 days it is impossible to obtain preparations suitable for study [6]

(3) that bone marrow is the only tissue where necrobiotic cell changes are always encountered (loss of nuclear turgor karyolysis karyorrhexis pyknosis karyomegalia and giant cell formation) and that for this reason Hueck[7] regards it together with lymphoid tissue as an organ of physiologic inflammation

(4) that the lineages of cell derivation given by various theories are arbitrary differ widely and lack proofs (Stohr[8])

(5) that the bone marrow very often behaves paradoxically becoming hyperplastic in *severe anemias and aplastic in leukemias*

(6) that like lymphoid tissue and especially thymus it involutes a process which begins at puberty

(7) that myeloid transformation has been recorded in many tissues (recently in the adrenal cortex[9]) and that such transformation is accompanied by degenerative changes

It cannot be insisted enough that what is most needed in the evaluation of research in this field is more respect for the facts of observation however discordant they are with the current theories and less respect for theories however widely accepted they seem to be

The object of the present work is striated muscle tissue in activity studied both in the living state and in histological material more precisely histolytic processes in the intact and traumatized contractile substance Muscle histolysis has been known as instantaneous process for over a century [10, 11] It can result in a complete liquefaction of the contractile substance or in the discharge of free sarcomeres (Bowman s disks) processes which although described have not been explained

The discharge in the intact muscle occurs in the form of single muscle columns or in that of transverse bands (cf figures in following communication) In traumatized or impaired muscle it *becomes massive and stationary and much more evident*

Apart from my own observations I was able to ascertain that many distinguished histol

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† Bibliographic references will be found in the following communication

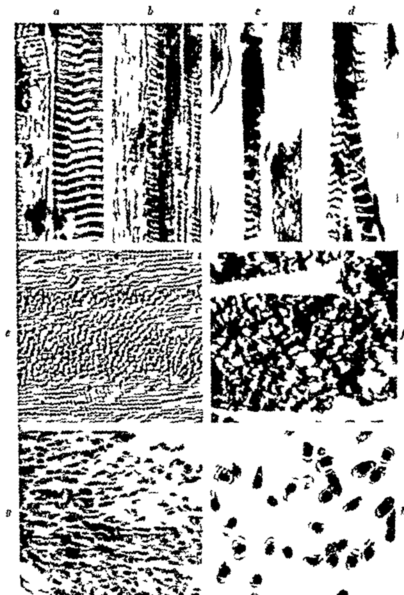


FIG. 1.—*a–d*, Stages in the transformation of a single muscle column (*a*, *b*, perfused muscle; *c*, *d*, muscle lesioned by heat); *e*, hemorrhages in a muscle injured by cold (phase contrast, unstained section; note the staircase pattern in the hemorrhage and in the muscle); *f*, perfused muscle, disintegration into bell-shaped elements containing glassy bodies in their concavities (in *f* mammalian muscle); *g*, rel[illegible] cross-striated erythrocytes in [illegible] muscle injured by injection of brilliant cresyl blue solution; *h*, blood film of the [illegible] (Triturus cristatus) smear from heart, transverse disks in some erythr[illegible] erythroplastids

siglo[10, 14] que la histólisis muscular es un proceso instantáneo Puede dar como resultado una completa licuefaccion de la substancia contráctil o la descarga de sarcomeros desprendidos (discos de Bowman)

Esta descarga en el musculo intacto se presenta en forma de columnas musculares simples o en la de bandas transversas (ver las figuras) En el musculo traumatizado o lesionado se hace masiva estacionaria y mucho más evidente

Fuera de mis propias observaciones he comprobado que muchos distinguidos histólogos no han podido diferenciar las columnas musculares y bandas transversas que sufren el cambio de capilares portadores de eritrocitos o la liberación masiva de la hemorragia

La hipótesis de trabajo basada sobre estas observaciones es como sigue la formacion de la sangre es el resultado de la histólisis igual que en los islotes sanguíneos embrionarios La histólisis de reemplazo normal y continua es la fuente de nuevos capilares y su contenido celular El mismo proceso desorganizado por el trauma o la enfermedad lleva a extravasaciones cambios inflamatorios deposición de componentes tisulares anormales El desecho celular (más abundante si el proceso es patológico) es depositado en el tejido linfoide y mieloide Estos no son considerados como los puntos de formación sino como los cementerios del desecho celular y al mismo tiempo fuentes de materia prima

Por lo tanto segun este concepto la hemopoyesis es de ubicacion intravascular de naturaleza histolítica y es citomórfica en sus resultados

II communication 2

Experimental and Comparative Documentation of the Cytomorphic Theory of Hemopoiesis

STANISLAW H WAJDA*

To put to experimental test the working hypothesis given in the preceding communication several procedures were adopted

(1) Portions of a working muscle (for instance diaphragm) were excised intact and traumatized *ex situ* then fixed immediately or after a delay ranging from less than a minute to five minutes In such conditions any pronounced cytomorphic changes could be reasonably regarded as the result of the trauma It can be stated that the histolytic phenomena can set in instantaneously and the cellular changes involved present the characteristics not of a slow transformation but those of a cell catastrophe

(2) Muscles traumatized *in situ* and excised for study after a delay ranging from ½ hour to a few days presented massive and stationary histolytic changes (discoid disintegration) much more disorganized and at the same time much more obvious The released discoid elements are indistinguishable from erythrocytes

The traumas used were of diverse nature mechanical (crush compression) thermal (heat cold) or chemical (various irritants) also the effects of ischemia of short duration have been studied The trauma is not regarded as a specific hemopoietic stimulus but the tissue reaction which it brings about and furthermore disorganizes makes the phenomenon more accessible for detailed cytologic analysis

(3) In another two series of experiments the muscles were thoroughly perfused *in situ* excised and stimulated either by short immersion in Tyrode s solution at 40 C or by electrical stimulation (diaphragm phrenic nerve preparation)

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The discoid disintegration thus induced did not differ from that observed in non perfused muscles. The bell shaped elements with glassy bodies in their concavities were a common finding (cf Fig 1f). Their staining properties with various stains were again identical with those of erythrocytes.

(4) Studies performed on isolated mammalian and amphibian living muscle fibers (chemical stimulation with lactic acid, potassium chloride, adenosine triphosphate and acetylcholine solutions, micromanipulation) confirmed the rapidity of the change.

(5) The comparative aspects appear to be by far the most important. The nucleated erythrocytes of lower vertebrates have an unmistakable morphology and staining characteristics. For reasons discussed by Helmke[16] and Monné and Slautterback[16] the Azan stain gave the best results.

Muscle histolysis in lower vertebrates—in contrast to the changes in the mammalian striated muscle—is preceded by an extremely massive nuclear proliferation in the form of multiple amitoses (meroamitoses of Thomas[17]). This proliferation is well known from earlier descriptions.[8, 18, 20] It results in the release of nucleated elements, the morphology and staining properties of which are, as in mammals, identical with those of erythrocytes. In some cases, however, the released cells are clearly cross striated.

(6) The study of fresh blood preparations of lower vertebrates, particularly with phase contrast, reveals often enough 4–6 *distinct transverse disks in the cytoplasm of erythrocytes* as also recorded by Barer.[21] The transverse bands can occasionally be seen also in stained smears. They remind one of the cross striated fibrils described in erythrocytes of the salamander by Meves.[22]

The Californian urodele Batrachoseps attenuatus, especially studied by Emmel,[3] has about 90 per cent of non nucleated erythroplastids, a fact which Emmel regarded as the result of cytoplasmic segmentation of the erythrocyte. This species seems to constitute an interesting "missing link" in the phylogeny of the mammalian erythrocyte.

Comparative studies show the inescapable fact that the striated muscle in physiologic histolysis undergoes a cytomorphic transformation into erythrocytes. The intimate relationship between myoglobin and hemoglobin is well known; myoglobin may therefore represent a unit structure of which four are combined to form the hemoglobin molecule.[25]

The hypothesis presented here to explain this fact might be wrong. It falls far outside the framework of conventional theories, but so does the fact.

The full results of this investigation will be published elsewhere.[26]

Documentacion Experimental y Comparativa de la Teoria Citomorfica de la Hemopoyesis

A fin de someter a prueba experimental la hipótesis de trabajo dada en la comunicación precedente se aplicaron varios procedimientos.

(1) Se extrajeron intactas y se traumatizaron *ex situ* porciones de un musculo activo (por ejemplo del diafragma) que fueron fijadas inmediatamente o después de una demora de menos de un minuto hasta de cinco minutos. En estas condiciones cualquier cambio citomórfico pronunciado podía ser considerado razonablemente como resultado del trauma. Puede afirmarse que los fenómenos histolíticos se presentan instantáneamente y los cambios celulares producidos no presentan las características de una transformación lenta sino la de una catástrofe celular.

(2) Los musculos traumatizados *in situ* y extraídos para su estudio después de un lapso de ½ hora hasta varios días presentaban cambios histolíticos masivos y estacionarios (desintegración discoide) mucho más desorganizados y al mismo tiempo mucho más evidentes. Los elementos discoides liberados son indistinguibles de los eritrocitos.

Los traumas usados fueron de diversa naturaleza: mecánicos (compresión), térmicos (calor, frío) o varios irritantes químicos; también se han estudiado los efectos de la isquemia de corta duración. El trauma no es considerado como un estímulo hemopoyético específico sino que la reacción tisular que el mismo provoca y además desorganiza hace al fenomeno más accesible para un análisis citológico detallado.

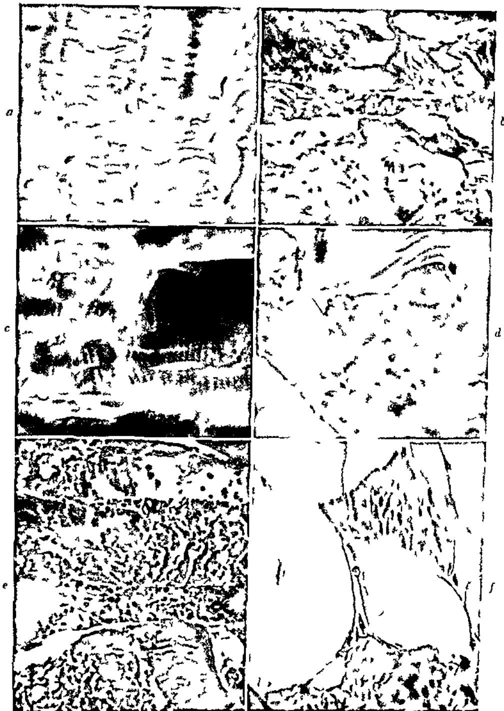


FIG. 2.—*a* Histolysis in the form of transverse bands (mammalian muscle); note their continuity with capillaries. *b* identical histolysis in a perfused mammalian muscle; note the empty vessels below. *c* massive histolysis in the mammalian muscle (high power). *d* massive histolysis (cell release) in the amphibian muscle. *e* massive discoid disintegration in 4 muscle fibers (mammalian muscle). *f* massive cell release in an amphibian muscle fiber. Compare the staining of released cells with that of erythrocytes below.

The specimens of mammalian muscles stained with iron hematoxylin of Hansen and modified Mallory stain; those of amphibian muscles with "Azan" of Heidenhain.

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II communication 3

The Content of Desoxyribonucleic Acid in Normal and Leukemic Myeloid Cells in Man

P INTROZZI*

The author presents the results obtained from spectro photometric analysis of the desoxyribonucleic acid content in nuclei of normal and leukemic granulocytic cells
These studies show that both normal and leukemic cells have the same amount of desoxyribonucleic acid This would be added evidence for differentiating leukemic cytopoiesis

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(3) En otras 2 series de experimentos los musculos fueron íntegramente perfundidos *in situ* extraídos y estimulados por una corta inmersion en solución de Tyrode a 40 C o bien por medio de estimulación eléctrica (preparación de diafragma—nervio frénico) La desintegracion discoide así provocada no difirió de la observada en musculos sin perfundir Los elementos en forma de campana con cuerpos vítreo en sus concavidades fueron un hallazgo comun (ver Fig 1*f*) Sus propiedades de coloracion con distintos colorantes fueron también idénticas a las de los eritrocitos

(4) Los estudios efectuados sobre fibras musculares vivas aisladas de mamíferos y anfibios (estimulacion química con soluciones de ácido láctico cloruro de potasio trifosfato de adenosina acetilcolina micromanipulación) confirmaron la rapidez del cambio

(5) Los aspectos comparativos parecen ser por lejos los más importantes Los eritrocitos nucleados de los vertebrados inferiores tienen una morfología y características de coloración inequívocas Por las razones discutidas por Helmke[15] y Monné y Slautterback[16] la coloración de Azan dio los mejores resultados

La histólisis muscular en los vertebrados inferiores—en contraste con los cambios en el musculo estriado de los mamíferos—va precedida por una proliferacion nuclear extremadamente masiva en forma de amitosis múltiples (meroamitosis de Thomas[17]) Esta proliferación es bien conocida por descripciones anteriores[18 19 20] Da como resultado la liberación de elementos nucleados cuya morfología y propiedades de coloración son como en los mamíferos idénticas a las de los eritrocitos En algunos casos sin embargo las células desprendidas son claramente estriadas

(6) El estudio de preparaciones de sangre fresca de vertebrados inferiores especialmente con contraste de fases revela muy frecuentemente 4 6 discos transversos claros en el citoplasma de los eritrocitos según registrara también Birer[21] Las bandas transversas pueden observarse ocasionalmente asimismo en extendidos coloreados Recuerdan a las fibrillas estriadas descriptas por Meves[22] en los eritrocitos de la salamandra

El urodelo californiano Batrachoseps attenuatus estudiado especialmente por Emmel[23] tiene alrededor de un 90% de eritroplástidos anucleados hecho que Emmel considero como resultado de la segmentacion citoplásmica del eritrocito Esta especie parece constituir un interesante eslabon perdido en la filogenia del eritrocito del mamífero

Los estudios comparativos demuestran el hecho ineludible de que el musculo estriado en histolisis fisiológica sufre una transformacion citomorfica en eritrocitos La íntima relación entre la mio y la hemoglobina es bien conocida[2] por lo tanto la mioglobina puede representar una unidad estructural de la que cuatro partes se combinan para formar la molécula de hemoglobina [25]

La hipótesis presentada aquí para explicar este hecho puede estar equivocada Cae fuera de la esfera de las teorías convencionales pero tampoco entra en ellas el hecho mismo

Los resultados completos de esta investigación serán publicados en breve[26]

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II communication 5

Histochemical Investigation of the Basophilic Granules

JAN TURCHINI and L KHAU VAN KIEN*

Study of the basophilic granulation has nowadays been continued in the rat Mastzellen based on the relationship between the Mastzellen granules and heparin Histochemical investigations on this last substance have determined its various constituents after fixation (with Carnoy s liquid for example) and inclusion susceptible of keeping the granulations in situ

Apart from the usual procedures the principal techniques used were those of sodium plumbite and barium salts to determine the sulfuric compounds and the fluorine reaction to identify the glucosamine

These reactions have been performed during the course of the experimental increase and decrease of the granulations and the number of Mastzellen

INVESTIGACIONES HISTOQUÍMICAS SOBRE GRANULACIONES BASÓFILAS

El estudio de estas granulaciones ha sido actualmente proseguido en los mastzellen de la rata

Sabiendo las relaciones existentes entre las granulaciones de los mastzellen y la heparina esta última substancia ha sido investigada histoquímicamente determinando sus diversos constituyentes después de haber precisado los modos de fijación (fijación por el líquido de Carnoy por ejemplo) y de inclusión suceptibles de conservarlas in situ

Independientemente de las técnicas usuales las principales reacciones practicadas han sido la del plumbito de soda y de las sales de bario para determinar los compuestos sulfúricos y las reacciones de los fluorones para identificar la glucosamina

Estas reacciones han sido practicadas por otra parte durante el curso del aumento o disminución experimental de las granulaciones y del numero de los mastzellen

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II communication 6

El Sistema Reticulo Endotelial del Bazo en Estado Normal y Patológico

M POLAK y G BOMCHIL†

La rica bibliografía existente sobre patología del sistema reticuloendotelial está basada salvo rarísimas excepciones en las imágenes microscópicas obtenidas con el empleo de técnicas comunes que no nos parecen suficientemente demostrativas En este trabajo planteamos un doble alegato

(a) En contra de las afirmaciones de que el sistema retículoendotelial es un estado funcional de un sector del tejido conjuntivo sostenemos que este sistema es una verdadera entidad morfológica con propiedades funcionales específicas y

(b) Que la técnica específica descrita por Río Hortega sabio español que tuvimos el privilegio de contar en nuestro país durante los ultimos cinco anos de su vida es imprescin

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from that of tumors in general because tumor cells contain in their nuclei an increased amount of desoxyribonucleic acid as compared to the same elements in normal tissues

La Contribución de la Citoquímica Hematológica al Problema Patogenético de las Leucosis Estudio Comparativo del Contenido Nuclear en Acido Desoxiribonucleico de las Células Mieloides Normales y Leucémicas

El autor expone los resultados que se obtienen mediante un análisis espectrofotométrico del contenido en ácido desoxiribonucleico de los nucleos de las células granulocíticas normales y leucémicas

De estos datos resulta que el contenido en a d n de los nucleos de las células mieloides normales es perfectamente igual a aquellos de los elementos leucémicos sería esto otro criterio de orden citoquímico que parece contribuír a diferenciar la citopoyesis leucémica de aquellas tumorales en general porque las celulas tumorales contienen en sus nucleos una cantidad de a d n significativamente superior a aquellos elementos homólogos de los tejidos normales

II communication 4

Changes in the Granulations of Neutrophilic Polymorphonuclears during the Course of Their Digestion of Phagocytized Bacteria

R ROBINEAUX and J FREDERIC*

By the use of high speed microcinematography the authors have studied in phase contrast and with histochemical methods the significance of the granulations of neutrophilic polymorphonuclears They have observed in this way the decrease in number and size and the disappearance of the specific granulations of these cells after phagocytosis of different types of bacteria The analysis of these films has allowed the establishment of a relationship between these granulations and the digestion vacuoles

A specific role can be given to the granulations of the neutrophiles in the cytophysiology of the polymorphonuclear cells

Estudio de la Relación entre las Granulaciones Específicas de lo Polinucleares y la Digestión de los Gérmenes que ellos Fagocitan

Los autores han estudiado con la microcinematografía acelerada en contraste de face y con los métodos histoquímicos la significacion de las granulaciones de los polinucleares neutrófilos

Ellos pueden observar la disminucion del numero y tamano y ver la desaparicion de las granulaciones específicas de estas células después de la fagocitosis de diversos gérmenes

Los análisis de estos films permiten establecer la relacion existente entre estas granulaciones y las vacuolas de digestión de los gérmenes fagocitados Parece ser que debe acordárele un papel preciso a las granulaciones de los neutrofilos en la citofisiología de los polinucleares

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the same manner as cancer serum we cultivated normal chick and rat embryo fibroblasts and treated them for 20 days with Hodgkin's serum A larger growth area was observed than in controls treated with normal serum with more numerous and definitely atypical mitoses

Cultures treated with Hodgkin's serum for 20 days were grafted into an adult hen's subcutaneous tissue 7 days later the formation of a hard capsulated nodule that grew until the 15th day was noticed Histological examination showed the presence of small round cells of the hematic series with abundant eosinophils Binucleated cells and cells with nuclei undergoing amitotic division in the process of apparent transformation into Sternberg's giant cells appeared in some of the nodules

ACCION DEL SUERO DE HODGKIN SOBRE LA CELULA NORMAL CULTIVADA IN VITRO

En una serie de trabajos anteriores hemos estudiado como las células normales cultivadas in vitro pueden adquirir caracteres patológicos al ser cultivadas en presencia de suero de portadores de tumores Con el fin de aclarar si el suero de portadores de enfermedad de Hodgkin se comporta en la misma forma que los sueros cancerosos cultivamos fidroblastos normales embrionarios de pollo y de rata y los repicamos durante 20 dias con suero de Hodgkin Se observó una mayor área de crecimiento respecto del control tratado con suero humano normal aumento del numero de mitosis y mitosis netamente atípicas

Los cultivos tratados con suero de Hodgkin durante 20 días fueron injertados en el subcutáneo de gallina adulta a los 7 días del injerto se observó la formacion de un nódulo duro encapsulado que aumentó hasta los 15 días El examen histologico mostro la presencia de pequenas células redondas de la serie hemática con abundantes eosinofilos En algunos de los nódulos aparecen algunas células binucleadas o con nucleo en division amitotica en vías de transformarse en células gigantes de Sternberg

II communication 8

Cytochemistry of Hemoglobin New Results on Microspectrographic Investigation of Intracellular Hemoglobin

SERGIO DE CARVALHO* and MAURICE F H WILKINS†

Using a microspectrographic technique with reflecting optics and narrow bands of the continuum (4047 and 2650 A) we have obtained pictures of cells (unfixed erythroblasts) showing different absorption patterns the first wave length (in the Soret band) is specific for heme absorption and allows us to demonstrate the presence of intranuclear heme granules through various stages of maturation of these cells This confirmed previous results arrived at by cytochemical methods The presence of nucleic acids has been demonstrated in neutrophil granulations The significance of intranuclear heme is discussed

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This work has been done in the Institute of Biophysics University of Brazil Rio de Janeiro and was supported by a grant from the National Research Council of Brazil

dible en el estudio de los componentes celulares del sistema retículo endotelial normal y patológico

Estas células repartidas en los órganos de la economía humana donde aparecen impregnadas de negro *con la técnica argéntica de* Pío Hortega tiene formas variadas y variables se disponen libremente o anastomosadas y en el bazo humano forman el citoretículo de los cordones de *Billroth tapizan los sinusoides* y son relativamente escasas en los folículos de Malpighi

Los estudios *realizados permiten demostrar*

(1) La posible evolucion fibroangioblástica y hemopoyética del sistema retículoendotelial

(2) Entre otras cosas demuestra definitivamente el origen retículoendotelial de los folículos tuberculosos y *sarcoidosicos* y *de las células* de Sternberg y de Gaucher

(3) Nuestras imágenes aun no senaladas en las esplenomegalias esclerocongestivas y en la purpura trombocitopénica que dejan abiertos caminos para nuevas interpretaciones etiopatogénicas

(4) La comparacion de las *imágenes obtenidas por medio de* este metodo en cortes histologicos y las que se observan en los extendidos de punciones esplénicas obtenidas del mismo material demuestran las grandes *limitaciones de este ultimo método* para el estudio de las células retículoendoteliales

THE RETICULOENDOTHELIAL SYSTEM OF THE NORMAL AND PATHOLOGIC SPLEEN

Rio Hortega s *technique of selective silver impregnation* of the cells of the reticulo endothelial system (R E S) differentiates these clearly from other splenic cells and has allowed us to study the histopathology *of some splenopathies*

In the sclerocongestive splenomegalies (Banti) hypoplasia and irregular shape of the R E S cells are found In idiopathic thrombocy*topenic purpura extrafollicular hyperplasia* of the R E S and marked intrafollicular hypoplasia are found In the acute leukemias and erythremias a hyperplasia or hypertrophy of the cells of the R E S *is observed contrast*ing with the findings in the chronic forms Diverse forms of change are described in reticulo endotheliosis and other splenopathies This method is of value in the study of R E S cells in splenic puncture

The systematic use of Rio Hortega s method is indicated for hematologic histopathology granted the special significance of the R E S in the physiopathology of the hematopoietic organs

II communication 7

Action of Hodgkin's Serum on Normal Cells Cultivated "in Vitro"

EUGENIA S de LUSTIG* and FABIO SACERDOTE†

In previous papers we have considered the means by which normal cells cultured in vitro acquire morbid characteristics when cultures in media containing serum from tumor cases In order to *discover whether serum from sufferers of Hodgkin s disease behaves in*

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Part III

Etiology and Treatment of the Leukemias

Etiologia y Tratamiento de las Leucemias

Citoquimica de la Hemoglobina Nuevos Resultados de la Investigacion Micro espectrografica de la Hemoglobina Intracelular

Mediante el uso de una técnica micro espectrográfica con reflecciones ópticas y bandas estrechas entre 4 047 y 2 650 A hemos obtenido imágenes de células (eritroblastos no fijados) mostrando distintos esquemas de absorcion la primera longitud de onda (en la banda de Soret) es específica para la absorción del heme y nos permite demostrar la presencia de gránulos de heme intranucleares en diferentes estados de maduración de estas células Esto confirma los hallazgos similares previos usando los métodos citoquímicos Se ha demostrado tambien la presencia de ácidos nucleicos en las granulaciones de los neutrófilos Se discute la significación del heme intranuclear

La leucemia aguda afecta más al sexo masculino que al femenino 58 9 por 41 1%, sobre todo antes de los 20 anos (2 x 1) a partir de esta edad la proporción es sensiblemente igual (cuadro 1)

2 *Clasificacion de las leucemias agudas*

	C so	%
(a) Segun el numero de leucocitos		
Con leucocitos normales (5 500 a 8 000 X mm ³)	14	9 05
Con leucopenia (5 500 a 600 X mm ³)	45	29 03
Con leucocitosis (8 100 a 50 000 X mm ³)	56	36 12
Con hiperleucocitosis (51 000 a 697 000)	40	25 80
(b) Segun la cantidad de blastos		
Forma leucémica (más 10% de blastos generalmente + de 2 000 leuc)	137	87 82
Forma subleucémica (menos 10% de blastos gener menos 2 000 leucocitos)	16	10 31
Forma aleucémica (0% de blastos y variable de leuc)	3	1 87
(Nota dos mieloblásticas y una linfoblástica)		
(c) Según la calidad de los blastos		
Reticuloendotelial (tipo Letterer Siwe)	3	1 77
Hemocitoblástica (indiferenciada)	7	4 13
Hemocitoblástica (linfosarcomatosa)	7	4 13
Plasmacelular	1	0 59
Linfoblástica	65	36 8
Mieloblástica	84	48 9
Promonocítica	6	3 54
(Ver cuadro 3 y microfotografias 2 3 4 5 6 7 y 8)		

3 *Polimorfismo celular*

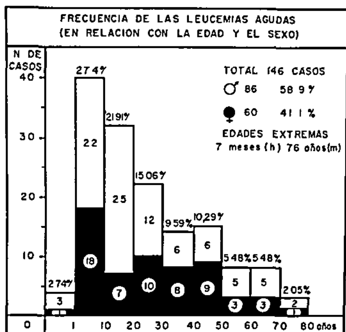
Llama la atención el gran polimorfismo celular de las leucemias agudas especialmente de la mieloblastica como lo han hecho notar entre otros Moeschlin y Rohr [2] El mieloblasto con los caracteres morfológicos que describiremos a continuación es el elemento mas frecuente pero generalmente se asocia a hemocitoblastos promonocitos monoblastos y aun promielocitos en proporción muy variable De los 84 casos 14 fueron exclusivamente a mieloblastos (inclusive micromieloblastos) 20 a mieloblastos con hemocitoblastos de Ferrata (mieloblastos agranulosos de Naegeli) 17 a mieloblastos-hemocitoblastos promonocitos 25 a mieloblastos y promonocitos 2 a mieloblastos y promielocitos 3 a mieloblastos hemocitoblastos promielocitos 2 a mieloblastos monoblastos promonocitos y 1 a promielocitos con uno que otro mieloblasto

En la leucemia linfoblastica el polimorfismo es menor en 24 casos solo se *encontro linfoblastos como elemento patológico en 22 linfoblastos y hemocito*blastos en proporción muy variable en 8 linfoblastos hemocitoblastos y prolinfocitos en 10 linfoblastos y prolinfocitos y en 1 prolinfocitos con uno que otro linfoblasto

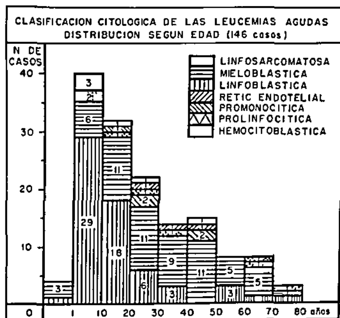
4 *Evolucion clínica*

Para establecer la evolucion solo se considero aquellos casos en total 95 en que ésta se pudo establecer con exactitud y que no estuvieron sometidos a tratamientos como la substitución sanguínea los antifólicos el ACTH y la cortisona que suelen prolongar apreciablemente el curso de la enfermedad en cambio se incluyó los que recibieron transfusiones y antibióticos cuyo efecto sobre la dura

Cuadro 1



Cuadro 2



año (el número tan reducido de casos por debajo de año no nos permite ser más categóricos)

La forma hemocitoblástica a elementos indiferenciados y en diferenciación (parcial) linfosarcomatosa especialmente esta última también es mucho más frecuente en el niño y adulto joven en cambio la promonocítica tiene la misma distribución que la *mieloblástica* de la que generalmente no constituye más que una diferenciación morfológica (cuadro 2)

forma promonocítica es semejante a la mieloblástica que en la forma hemocitoblástica es frecuente una discreta esplenomegalia y ocasional la micropoliadenopatía y la hepatomegalia y que en la forma linfosarcomatosa los hallazgos físicos son similares a la leucemia linfoblástica aunque los ganglios tienen tendencia a ser más localizados y de mayor tamaño agregando e a veces, infiltraciones cutáneas del seno amígdalas testículos etc

B Naturaleza de las Leucemias

1 *Consideraciones generales*

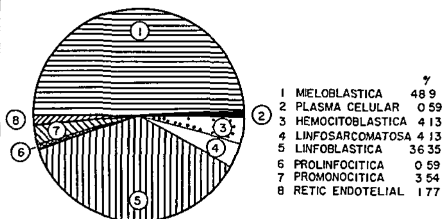
Las tres tendencias etiopatogénicas actuales son la infecciosa la neoplásica y la que considera a la leucemia como una enfermedad de la adaptación

Naturaleza infecciosa Craigie y Bennett[2] en 1845 consideraron las leucemias como supuraciones sanguíneas o piemias al identificar erroneamente los leucocitos como pus Virchow (1845–46) corrigió dicho error y describió la enfermedad —como una entidad especial—con el nombre con que actualmente se la conoce Ehrlich (1866) reconoció el carácter sistematizado de la leucemia linfógena y sostuvo su origen infeccioso o que Naegeli hizo extensivo a la leucemia mieloblástica (1900)

Los argumentos en que se apoya esta teoría poco sólidos en su mayoría refutables son los siguientes apariencia clínica a menudo infecciosa, hallazgo frecuente de gérmenes en la sangre comprobación de pequeños brotes epidémicos y carácter infeccioso a virus de la leucemia de las gallinas Sin embargo no se ha demostrado su contagiosidad ni trasmisibilidad dentro de la especie humana tanto trasplacentaria como por inyecciones de sangre o plasma[4, 5] ni tampoco a los animales de experimentación La calidad de las células y su trastorno de maduración (hiatus) no se observa en ninguna infección conocida Además no ha sido posible homologarla a la leucemia de los pollos cuya similitud aparente con ella constituye uno de los argumentos de más peso a favor de su naturaleza infecciosa

La etiología cancerosa fué planteada por Babes (1902) Banti (1905) y Ribbert (1907) quienes la consideraron como sarcomas de rápida difusión y so tenida por Mallory (1914) para la leucemia linfática que incluyó entre los linfoblastomas Actualmente cuenta con numerosos adeptos Moeschlin Rohr Heilmeyer Rosslé Burchenal Wiseman etc

Los argumentos a favor de esta teoría mucho más numerosos y más demostrativos son (a) carácter incurable de la enfermedad curso fatal rápidamente progresivo salvo remisiones espontáneas[6, 7] o determinadas por diversas terapéuticas que por lo demás también se observan ocasionalmente en los cánceres aun con metástasis (b) Ausencia de cuadro infeccioso en los casos diagnosticados precozmente éste como en la agranulocitosis constituye una complicación subordinada a la baja de las defensas (c) Aparición en circunstancias que determinan o favorecen la producción del cáncer como los traumatismos con fracturas óseas intoxicaciones por benzol[8] radiaciones Röntgen alquitrán dibenzatraceno metilcolantreno anilinas y estrógenos substancias que aumentan la incidencia de la leucemia de las ratas (Furth) (d) Asociación a cánceres de la piel y mamas



Cuadro 3

cion de la leucemia es mucho menos notable El promedio de evolución fué similar en los diferentes tipos de leucemia y fluctuo entre 3 meses y 3 5 meses La duracion mínima aparente fué de 4 dias y la maxima de 1 ano 3 meses con una remision espontánea Es interesante consignar que 5 casos de leucemia mieloblastica evolucionaron aparentemente en 4 a 20 dias que un caso de leucemia linfoblastica controlada por mielogramas repetidos duro 3 años durante los cuales tuvo dos remisiones espontaneas y termino en linfosarcomatosis (leucosarcoma) que en total—con los dos referidos—hemos observado tres casos de remision espontanea en 173 leucemias una de ellas coincidio con un tratamiento con estreptomicina instituído por una adenitis tbc caseosa cronica en el segundo caso también coexistio una adenitis tbc caseosa aguda que mejoró en forma rapida y espontanea En el tercero la remision clínica completa y la medular (solo persistio *uno que otro blasto) mantenidas durante 6 meses fueron determinadas* por la regresión terapéutica total de una crisis de anemia perniciosa típica concomitante

5 *Semiologia ganglionar esplenica y hepatica*

La leucemia linfogena determina con mayor frecuencia un aumento apreciable del bazo (72%) ganglios generalizados (65%) y hepatomegalia (52%) en la leucemia mieloblastica fué en cambio mas frecuente y de mayor consideracion la hepatomegalia (42%) que la esplenomegalia (37%) y la micropoliadenomegalia (solo 15%) En cambio adenopatías localizadas cervicales generalmente traqueobronquicas o lumboaorticas a veces de naturaleza tuberculosa (caseosa) se encontro en 39% contra 15% de la forma linfoblastica La casuística reducida de leucemias promonocíticas hemocitoblasticas y linfo arcomato as no permite dar porcentajes pero en lineas generales puede establecerse que la semiología de la

de las células leucémicas y normales a diferencias de las células cancerosas en que se halla aumentado, sin embargo dicho aumento ha sido comprobado en los núcleos en actividad mitósica tanto en estudios cualitativos de la reacción de Feulgen efectuados por Polli [7] como cuantitativos hechos por Thorell en la especie humana[8] y por Peterman y Mason en la leucemia experimental [7]

La naturaleza neoplásica de las células leucémicas no involucra una determinada etiología y por lo tanto no excluye

(a) El origen viral que también ha sido invocado para el cáncer y al parecer comprobado por Peyton Rous[9] en el sarcoma de las aves no ha sido demostrado para la leucemia de los mamíferos ratas y cuyes que se trasmiten por células viables solamente y menos aun para la humana que no es trasmisible Así el cuadro determinado por Magrassi Negroni y Tolu[10] al inocular en cuyes filtrados leucémicos acelulares y cuya naturaleza viral sugiere su reproducción en serie no sería absolutamente homogable a las leucemias ni aun al asociar agentes cancerígenos

Torrioli[11] quien también es partidario de la etiología viral sostiene que la autoglutinación de los hematíes que observo en las gallinas inoculadas con materiales leucémicos y que durante años por traspasos sucesivos han hecho mesenquimopatías letales es otro argumento a favor de dicha naturaleza Pero en las leucemias humanas la autoaglutinación no es un fenómeno frecuente

(b) Que la leucemia sea determinada por el desequilibrio entre una substancia antileucémica y otra estimulante o favorecedora de la leucemia como sostienen Bessis y Dausset Bierman y colabs [4]

(c) Que en su aparición o desarrollo participen como cree Miller [6] una deficiencia o un desequilibrio entre los llamados ácidos mielo y linfokéntricos aislados de la orina de la leucemia correspondiente y que experimentalmente reproducirían alteraciones histopatológicas de los órganos hematopoyéticos similares a las de la leucemia respectiva Normalmente estas substancias estimulan la multiplicación celular o hiperplasian el sistema correspondiente y maduran el opuesto De su equilibrio resultaría la normalidad del cuadro leucocitario

(d) No excluye tampoco la participación de un trastorno endocrino sobre todo hipofiso córticoadrenal muy posible si se considera la correlación existente normalmente entre dichas glándulas y el sistema hematopoyético La secreción corticoadrenal inhibe el tejido linfógeno y estimula el mielógeno su deficiencia se traduce por hiperplasia linfoide Así sucede en la suprarrenalectomía intervención que determina además un aumento de la trasmisibilidad de la leucemia en las ratas (Furth) Las hormonas corticotrópicas y córticoadrenales hipoplasian el tejido linfógeno estimulan la mielopoyesis y disminuyen la incidencia de la leucemia experimental

La disminución de la excreción urinaria de los 17 cetoesteroides en las leucemias tanto agudas como crónicas comprobada por Levin [1-3] Hanlon [13] Dobriner[14] y por nosotros mismos con Acevedo[15] también traduce una insuficiencia metabólica córticoadrenal que aunque no exclusiva de esta enfermedad podría tener una importante participación en su génesis y especialmente en su desarrollo o evolución como permite suponerlo el efecto favorable de los tratamiento con dichas

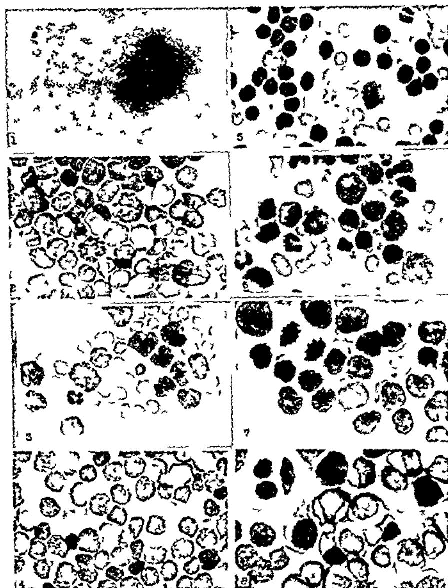
especialmente 2% en estadísticas extranjeras y 1.7% en la nuestra (dos cánceres del seno y uno de la lengua). La frecuencia no es por cierto tan demostrativa como la del Ca. gástrico en la anemia perniciosa. (e) Pasaje de la leucemia crónica mielogena a la aguda. El cuadro clínico y hematologico adquieren evidentes caracteres de malignidad, sobre todo la célula leucémica cuya morfología varía fundamentalmente. (f) Pasaje de la leucemia linfogena aguda al linfosarcoma y vice versa, por lo tanto coexistencia de ambos cuadros (leucosarcoma). (g) Existencia de formas malignas o tumorales de leucemia, como el cloroma y el linfosarcoma. (h) Similitud histopatologica de estos dos procesos a tal punto que suele ser muy difícil—y a veces imposible—su diferenciación. (i) Reproducción de la leucemia de los mamíferos (ratas y cuyes) no por filtrados sino con células viables intactas (Gasic y colaboradores). (j) Reproduccion experimental a voluntad en ratas y gallinas—con una misma cepa de células leucémicas—de sarcomas, leucosarcomas y leucemias linfogenas segun la vía de inoculacion (Furth, Richter, Mac Donald, etc.). (k) Produccion de leucemias por inoculación de microorganismos aislados de tumores epiteliales (Young) o con materiales o filtrados de canceres de endotelioma del pollo (Oberling y *colabs.*), spindle cells sarcoma (Parsons) etc., o de sarcomas o carcinomas—reversibles al efectuarse la reimplantación de ellos—con productos leucemicos.

Existen sin embargo algunos argumentos en contra como la sistematizacion d'emblée opuesta a la localizacion inicial de los tumores malignos y a su propagacion *por metástasis localizadas o nodulares. Sin embargo han sido descritas* leucemias localizadas o de origen local: piel, ganglios, médula ósea, etc. y además—ocasionalmente—como nosotros mismos lo hemos observado, invasion medular focal de la leucemia linfoblástica demostrables en los frotis como islotes compuestos de numerosos linfoblastos, rodeados de intensa hiperplasia eritroblástica. *Estas pequeñas zonas de metaplasia han pasado desapercibidas en el corte histo*logico. En todo caso, la rapida difusión de las leucemias o su aparente sistematización de comienzo, es un fenomeno inherente a la naturaleza misma de los órganos hematopoyéticos que aun no ha podido ser explicado satisfactoriamente.

Tampoco el metabolismo respiratorio y fermentativo de las células leucémicas apoyan su *naturaleza cancerosa, pues es similar al de los tejidos embrionarios para* las células inmaduras de la leucemia mielogena y las maduras e inmaduras de la linfógena, esto es, consumo de oxígeno elevado, de glucosa anaerobio fuertemente aumentado y aerobio bajo; en cambio los leucocitos maduros, tanto los de la sangre normal como los de la leucemia mielógena, tienen como los canceres, un consumo *de oxígeno bajo y un franco aumento de la glicolisis aerobia y anaerobia.*[2]

Estos resultados no son concluyentes y así Victor, Potter y Wintersteiner[3] concluyen que estas pruebas realizadas en los tejidos leucémicos no permiten establecer su etiología infecciosa o cancerosa. Aun mas, Hess[4] encuentra cambios metabolicos de tipo canceroso, aun antes de hacerse manifiestas las lesiones histopatologicas características *en los organos—especialmente en el hígado—de* las ratas intoxicadas con benzol.

Por ultimo Introzzi señalo recientemente en una comunicacion a este Congreso[5] que no existe diferencias en cuanto al contenido en ácido desoxiribonucleico



Microfotografías 1-8

reproductora o proliferativa de dichas células. El núcleo pasa a primer plano tanto en sentido fisiológico como morfológico, y es así como a veces la célula está constituida casi exclusivamente por el núcleo, especialmente los linfoblastos. La diferenciación protoplasmática es sólo esbozada y presenta ciertas particularidades morfológicas y estructurales que, sin ser propiamente de naturaleza neoplásica, facilitan la identificación y *clasificación de las células leucémicas* y su inclusión en los *diferentes* tipos consignados a continuación.

hormonas sobre el cuadro clínico,[17-20] las alteraciones hematológicas y medulares[21]

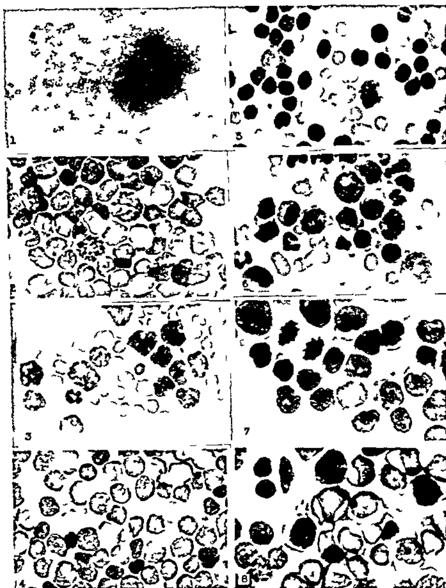
(e) Es posible además la participación de una predisposición genética, de un terreno ligado a la herencia como también se supone para el cancer sobre todo en las leucemias con gran incidencia familiar (Weiss y Decastillo) Es difícil explicar de otra manera la gran frecuencia de la forma linfogena en dichas circunstancias La leucemia humana no es hereditaria porcentualmente segun las leyes de la herencia como la de las ratas, se hereda más bien un terreno posiblemente ligado a una insuficiencia hipofiso corticoadrenal a veces congénita y similar al estado tímico linfático un estado de hipoadrenia congénita con hipomielia como sostiene Jimenez de Asua[18] Las leucemias pasarían a ser enfermedades de la adaptacion[16] [18] Este concepto actual precisa la vieja concepcion de Naegeli[19] que consideraba las leucemias como enfermedades determinadas por trastornos de la correlacion o de la regulacion de los organos hematopoyéticos debidos a perturbaciones funcionales irreversibles de las glandulas de secrecion interna

(f) La teoría granulocitolica de Aleksandrowics[9] no tiene fundamentos sólidos La leucemia mieloblástica y la agranulocitosis son dos entidades hematologicas muy diferentes La neutropenia granulocitica invocada como mecanismo de la leucemia por el autor no es constante gran parte de los casos con leucocitosis y especialmente aquellos con hiperleucocitosis suelen tener neutrofilia a veces acentuada El benzol que es granulocitolítico produce además de leucemias mieloblasticas agudas leucemias mielogenas cronicas (6) con gran aumento de los neutrofilos maduros Con el mismo derecho la eritrocitolisis de las anemias hemoliticas cronicas debería determinar eritremias

En contra de la naturaleza neoplasica de las leucemias agudas se ha invocado además (a) las remisiones espontáneas[1] poco frecuentes y temporales[2] pero éstas también se presentan ocasionalmente en los canceres aun con metastasis (observacion personal de metastasis cutanea de un Ca pulmonar) En cuanto a las remisiones terapéuticas basta recordar que el linfosarcoma p ej regresa completamente in situ con diversas terapias (b) La maduracion de las células leucémicas en los cultivos (Bichel) No queremos poner en duda esta aseveracion pero señalamos que las células neoplasicas inclusive las leucémicas suelen experimentar cambios morfologicos regresivos preferentemente nucleares (condensacion cromatínica) que podrían ser erroneamente considerados como debidos a maduracion Ademas la falta de pasaje de la forma aguda de leucemia a la cronica hace dudar de las posibilidades de maduración de las células de la leucemia aguda

2 *Cambios Morfologicos de las Celulas Leucemicas*

La célula leucémica (de la leucemia aguda) es sin duda un elemento anormal (ausente normalmente en los organos hematopoyéticos) inmaduro indiferenciado o rudimentariamente diferenciado[2] y atípico que se reproduce indefinida y anarquicamente formando enormes agrupaciones de aspecto uniforme o brotes similares a los cancerosos (Microfotografia 1) los que junto con las figuras de división mitosica y amitósica dan una clara idea de la sorprendente actividad



Microfotografías 1-8

reproductora o proliferativa de dichas células. El núcleo pasa a primer plano tanto en sentido fisiológico como morfológico y es así como a veces la célula está constituida casi exclusivamente por el núcleo, especialmente los linfoblastos. La diferenciación protoplasmática es sólo esbozada y presenta ciertas particularidades morfológicas y estructurales que sin ser propiamente de naturaleza neoplásica facilitan la identificación y clasificación de las células leucémicas y su inclusión en los diferentes tipos consignados a continuación.

(a) *Hemocitoblasto o blasto indiferenciado* (Microfotografía 2) Dimensiones muy variables desde 7–8 μ (microhemocitoblastos) a 16–18 Promedio 10 a 11 μ Forma redondeada Escaso protoplasma de basofilia tenue a veces mas claro alrededor del nucleo Este es grande (ocupa 4/5 partes de la célula) y de forma muy variable a menudo redondeado pero con escotaduras en sus bordes o surcos de variable profundidad en su superficie que aparece abollonada a veces lobulada El nucleo adquiere un aspecto voluminoso, macizo, pero su estructura cromatínica es finamente y uniformemente granular o reticulada, a veces con algunos engrosamientos basicromáticos Contiene 0 a 5 nucleos generalmente 2 o 3 pequeños o 1 grande Aquellos que presentan algunas granulaciones azurófilas o bastoncitos de Auer deben ser considerados como mieloblastos

Los hemocitoblastos linfosarcomatosos (Microfotografía 3) se caracterizan por atipias morfologicas nucleares más marcadas especialmente mayor tamaño multilobulación y variabilidad de la estructura cromática nuclear, y del numero y tamaño de los nucléolos

A veces es dificil y aun imposible diferenciar los hemocitoblastos de los linfoblastos pues se producen cambios regresivos de la cromatina nuclear que dan al nucleo del hemocitoblasto una apariencia de mayor madurez, y a la célula un aspecto linfoblastico

(b) Linfoblastos (Microfotografía 4) Mas pequeño que el hemocitoblasto en general Promedio 8 a 12 μ Forma redondeada u oval Protoplasma poco basofilo muy escaso a menudo reviste parcialmente el nucleo no es excepcional observar nucleos aparentemente desnudos Nucleo grande (ocupa 4/5 del area celular) de forma muy variable con tendencia a ser oval o poliédrico (con uno o dos bordes rectilíneos) La basicromatina forma un retículo mas condensado menos regular con engrosamiento en estrías y pequenos grumos ausencia de nucléolos o presencia de uno excepcionalmente dos a menudo destacado por la condensacion periférica de la basicromatina Con menor frecuencia que el hemocitoblasto presenta escotaduras y surcos que hacen irregular su superficie y dan idea de volumen

(c) Prolinfocito (Microfotografía 5) Aun mas pequeno 7 a 8 μ Redondeado Muy escaso protoplasma basofilo con frecuencia nucleos aparentemente desnudos de forma redondeada a veces algo escotado y superficie lobulada cromatina en trazos y grumos densos dispuestos con cierta regularidad (tablero de ajedrez) ausencia de nucléolos

(d) Mieloblasto (Microfotografía 6) En general de mayor tamano que los elementos anteriores 14 a 20 μ a veces mas excepto los micromieloblastos (8 a 10 μ) Forma redondeada u oval en su periferia o contorno se observan—a veces —pequenas formaciones redondeadas como apéndices u orejuelas El protoplasma es mas abundante ocupa 1/4 o 1/3 del área celular su basofilia es discreta y a veces es mas claro aun alrededor del nucleo Ocasionalmente hiperbasofilia protoplasmatica Contiene pequenas granulaciones azurofilas bien contrastadas en numero variable a veces bastoncitos de Auer (Microfotografia 7) (uno ocasionalmente varios) y vacuolas protoplasmaticas aun sobre el nucleo Este es de forma irregular ovalado reniforme escotado o lobulado abollonado La

basicromatina e granular o forma un retículo fino y uniforme Los nucléolos son muy variables en tamaño forma a veces y especialmente en numéro 0 a 8 generalmente 1 a 3 grandes cuando son escasos pequeños si son numerosos

(e) Monoblastos Poco frecuentes y de identificación a menudo insegura Es semejante al mieloblasto sobre todo en su morfología y estructura nuclear pero el protoplasma contiene gran cantidad de granulaciones finas pulverulentas especialmente alrededor del nucleo éstas ocupan con frecuencia solo parcialmente el protoplasma y estan separadas—a veces—de la parte agranulosa periférica por un contorno circular bien preciso En los bordes de esta célula suelen encontrarse las mismas prolongaciones amibiformes descritas en el mieloblasto y presentes también—a veces—en los promonocitos

El monoblasto agranuloso reticular es una célula muy rara y difícil de identificar como tal El elemento característico de la leucemia aguda monocítica es en realidad el promonocito

(f) Promonocito (Microfotografia 7) Dimensiones 14–18 μ como un monocito o algo más grande Protoplasma abundante como el mieloblasto pero con granulaciones similares a las del monocito normal afectando a menudo la disposición descrita en el monoblasto A veces alternan con ellas granulaciones azurófilas más netas en menor numero similares a las del mieloblasto entonces la diferenciacion con este elemento llega a ser imposible Creemos que ésto se debe a que por lo menos en circunstancias patologicas (también en la agranulocitosis) el monocito se deriva del mieloblasto de Ferrata (granuloso) Así se explicaria—para nosotros—la existencia de estos elementos aparentemente híbridos La diferencia principal con el mieloblasto estriba en el nucleo que ocupa 2/3 ó 3/4 de la célula es reniforme a veces escotado y aun dividido en dos por un profundo surco no tiene nucléolos o presenta sólo un esbozo de él Estos son los promonocitos mas corrientes y caracterizan la leucemia a monocitos tipo Naegeli que no sería mas que una variedad morfologica de la leucemia mieloblastica Los de origen reticuloendotelial constituyen la excepción y son propios de la leucemia a monocitos tipo Reschad Schilling [3] La microfotografia 8 consigna los plasmazellens inmaduros y los blastos linfocitoides unos con aspecto reticular y otros de linfocitos de Downey de una leucemia plasmacelular

Las atipias morfológicas que permiten identificar a las células leucémicas como cancerosas se refieren a la célula misma en general y al nucleo en especial a la forma de éste a su tamano estructura cromatínica figuras de division y nucléolos

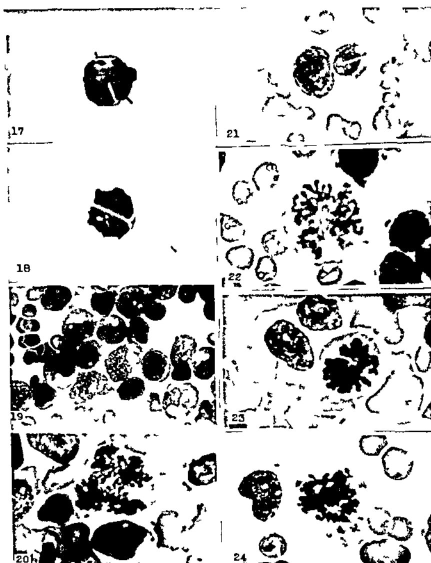
El tamano de la célula es extraordinariamente variable 6–8 micrones hasta 20 y 30 o más ocasionalmente a veces se encuentra en la médula elementos verdaderamente monstruosos de 50–60 μ que recuerdan a las células de los cánceres escamosos (Microfotografias 9 y 10) El polimorfismo celular es notable en las leucemias promonocíticas en las que a veces algunos elementos muy alargados recuerdan las células en cometa de la variedad de cancer precitada las fagocitosis simulando las células en ojo de pájaro o eyebird son excepcionales (Microfotografia 11) en cambio son mas frecuentes en los mieloblastos y promonocitos excrecencias protoplasmáticas como pseudopodios (Microfotogra



Microfotografias 9-16

fia 12) parecidas a las de las células de los canceres del epitelio de transicion de la vejiga urinaria y cérvico vaginal las vacuolas protoplasmáticas de los hemo citoblastos y mieloblastos (Microfotografia 13) que suelen observarse en los adenocarcinomas y a veces en los linfosarcomas y la fragilidad celular sobre todo protoplasmatica debido posiblemente a la rapida multiplicación de la célula Se traduce esta ultima por la presencia de nucleos casi desprovistos de proto plasma (linfoblastos hemocitoblastos células reticulares) o libres de él y en dife rentes grados de desintegracion constituyendo las placas reticulares o restos de Gumprecht de la leucemia linfógena (Microfotografia 5)

Los cambios morfo estructurales del nucleo tiene mucho mas valor como signos



Microfotografias 17-24

de degeneración neoplasica El tamano es muy variable aun en un mismo extendido 6 a 20 y en algunos casos 30 o más micrones especialmente en la médula ósea (casos de leucemia mieloblástica) Marcado polimorfismo nucleos redondeados ovales poliédricos arrinonados en trebol etc Aspecto macizo voluminoso (Microfotografia 14) a veces escotado y abollonado debido a surcos superficiales y profundos que estrangulan el nucleo y lo lobulan llegando aun a dividirlo en dos parcial o totalmente división amitósica a veces muy intensa en las diferentes variedades de leucemia (Microfotografias 15 16 17 18 diferentes fases de division amitósica)

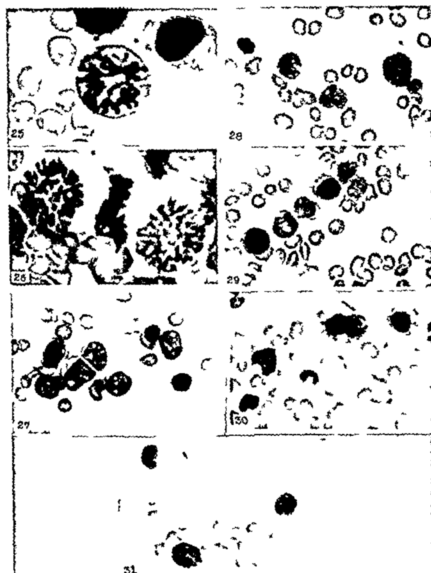
El nucleo en su crecimiento irregular e incontrolado exuberante se pliega

sobre sí mismo como si el espacio dentro de la célula disponible a su desarrollo se hiciera insuficiente. Son núcleos hipercromatínicos, proliferantes (Microfotografía 19) muy diferentes en su estructura a los núcleos estacionarios o prenecróticos [4] de las células normales y muy ricos en ácido desoxiribonucleico cuando se hallan en plena reproducción mitósica [7] o actividad proliferativa [4].

La reproducción celular por carioquinesis o mitosis es muy frecuente en las diferentes variedades de leucemia: en los centros hematopoyéticos a veces se encuentran 2 o 3 por campo microscópico (Microfotografía 26). En la sangre periférica suelen observarse cuando existe hiperleucocitosis. A menudo son atípicas como en el cáncer, pero ésto no es constante ni exclusivo de dichas enfermedades. En realidad su valor diagnóstico como signo de malignidad estriba en la frecuencia con que se encuentran, en la proporción que alcanzan en las leucemias agudas y a su extrema variabilidad. P. ej.: husos asimétricos, multipolaridad de los husos (Microfotografía 20), número desigual de cromosomas, dispersión de ellos que pueden quedar aislados y persistir al formarse el nuevo núcleo (Microfotografía 21), *disposición irregular de los cromosomas* (Microfotografía 22), deformación de ellos: generalmente se acortan y engruesan tomando aspecto de clavas (Microfotografía 23) y llegando aun ser granulares (Microfotografía 24), picnóticos, otras veces se bifurcan en sus extremos (cromosomas dendríticos—Microfotografía 25) o se tiñen irregularmente: a menudo se hipercolorean y toman aspecto monoliforme. Otras veces no se individualizan bien, parecen aglutinados (Microfotographia 26) y al separarse los husos quedan unidos por puentes o bridas.

Nucléolos.—De formas diferentes y muy variables en tamaño y número (Microfotografía 27). Su valor como signo de malignidad es superior al de las mitosis, sin embargo no son imprescindibles para identificar a la célula leucémica como neoplásica: con la estructura cromatínica y la morfología nuclear suele bastar. Su forma redondeada u oval sólo se modifica apreciablemente en los grandes nucléolos que suelen ser irregulares (Microfotografía 19). Su tamaño en cambio experimenta considerables variaciones: suele ser mucho más grande el *nucléolo aislado* (Microfotografía 28) *que los nucléolos múltiples considerados* individualmente, pero la masa nucleolar esté siempre aumentada, como lo ha establecido González Guzmán en sus índices nucleolares [5, 6]. Su número fluctúa entre 1 y 5 en los mieloblastos y hemocitoblastos, especialmente en la forma linfosarcomatosa de esta célula. Pueden llegar a 7 u 8, pero también pueden estar ausentes. Aproximadamente en el 30 % de los linfoblastos se encuentran un nucléolo, excepcionalmente dos; en el resto ausencia de nucléolos o a veces esbozo de ellos, como sucede también en los promonocitos (Microfotografía 7).

El extraordinario polimorfismo (Microfotographia 29), inmadurez o falta de diferenciación y atipicidad de las células, hace difícil su identificación y a veces imposible su clasificación correcta, que se basa en cierto parecido morfológico con los elementos progenitores normales de los centros hematopoyéticos: hemocitoblastos [33], mieloblastos, linfoblastos, etc. La célula más anormal, inmadura y atípica es la que establece el tipo de leucemia, el aumento de una determinada variedad de leucocitos normales maduros: linfocitos, neutrófilos, monocitos no



Microfotografías 25-31

puede constituir un signo de valor para identificar las células leucémicas y decidir el tipo de leucemia linfoblástica mieloblástica y promonocítica respectivamente si se considera la imposibilidad de madurar o diferenciarse de estos elementos

Que se trata de elementos anormales diferentes a aquellos con los que se les identifica se demuestra en las leucemias mielógenas crónicas agudizadas los mieloblastos del período de agudización son morfológicamente muy distintos a los de la fase crónica y semejantes a los de la leucemia mieloblástica démblée

(Microfotografías 30, 31). Se produce una substitución de una hiperplasia celular crónica sistematizada de los centros hematopoyéticos de tipo irritativo aparentemente benigna, pero potencialmente cancerosa (estado precanceroso de Heilmeyer), por una displasia celular maligna, cancerosa, de crecimiento tumultuoso, que se traduce en un curso clínico extraordinariamente rápido, progresivo, fatal a breve plazo.

La causa de ambos procesos puede ser la misma: la frecuente transformación de la forma crónica en aguda (20 a 25% según nuestra experiencia) y la existencia ocasional de *formas clínicas de transición inducen a suponerlo. Es posible que* la causa X de la leucemia crónica, al actuar persistentemente sobre los elementos hematopoyéticos acelerando su multiplicación y maduración, determine (por *agotamiento del principio de maduración*) la transformación cancerosa de ello exteriorizada por cambios morfológicos similares a los de las células de otros cánceres y que permiten identificarlas como tales.

En resumen: Las células de la leucemia aguda son morfológicamente elementos anormales, inmaduros y atípicos, de naturaleza maligna, cancerosa, ya que reúnen la mayoría de los cambios morfológicos que permiten identificar con seguridad las células cancerosas de los tumores malignos del resto del organismo, ya sean sarcomas o carcinomas (como ha podido apreciarse a través de la microcitografías de células cancerosas y leucemias proyectadas). Su maduración o mejor dicho su diferenciación esbozada o rudimentaria permite además clasificarlas—aunque no siempre con seguridad—por cierto parecido con los elementos progenitores de los centros *hematopoyéticos*, como para hemocitoblasto, para mieloblastos, para linfoblastos, etc. A las células hematopoyéticas les asiste el mismo derecho a transformarse en células cancerosas que a las de otros tejidos, y *cada célula progenitora puede dar origen a un determinado tipo de célula* neoplásica primitiva [7] que tiene algunas características físicas, morfológicas y fisiológicas del elemento normal que les ha dado origen, como ser granulaciones *en los mieloblastos y promonocitos, y posibilidad de pasar a la circulación*.

La célula progenitora se transformaría en célula cancerosa y continuaría multiplicándose indefinidamente sin madurar, debido a la acción de un agente causal X que requeriría para actuar la concurrencia de múltiples factores (ya a ello se debería su relativamente escasa frecuencia), unos intrínsecos, constitu*cionales o genéticos, endocrinos, especialmente hipofiso corticoadrenal*, nutritivos, y otros extrínsecos, físicos, químicos, etc., como ha sido señalado por Wintrobe.

CYTOMORPHOLOGICAL CLASSIFICATION OF ACUTE TYPES OF LEUKEMIA. MORPHOLOGIC CONSIDERATIONS ABOUT THE POSSIBLE NEOPLASTIC NATURE OF LEUKEMIC CELLS

After a short survey of the universal experience on the etiology of leukemia, the authors explain their own observations which are based principally on study of the morphology of leukemic cells in comparison with cancer cells of other organs and systems besides the hematopoietic. Their cytomorphological classification, coinciding in its general outlines with the one now universally accepted, shows by statistics the different varieties of acute leukemia, according to their morphostructural characteristics which permit identification of the various types of leukemic cells. They are registered by *colored microphotographs* together with several unusual morphological and nuclear types similar to those observed in cancer cells.

The authors stress the often insurmountable difficulty in establishing differences between pathological myeloblasts and promonocytes and occasionally between these latter and promyelocytes in the forms of acute leukemia usually considered as myelogenous (myeloblastic)

Stress is laid upon the abnormal and atypical character of these leukopoietic elements para myeloblasts para lymphoblasts of Naegeli and sometimes even para promonocytes which makes their identification difficult indeed

Besides reticulo endothelial leukemia or rather leukemic reticuloendotheliosis which occurs but seldom the authors distinguish two well-defined types acute lymphogenous leukemia (lymphoblastic) and acute myelogenous leukemia in its two varieties myeloblastic and promonocytic often difficult to differentiate (myeloblastic promonocytic leukemia) This latter type seems to be characterized by one common leukoblastic element frequently showing certain morpho structural peculiarities mostly protoplasmic that usually permit identification either as a myeloblast or as a promonocyte

However this differentiation is not always possible (leukoblasts not exactly differentiated) because the morphological characteristics may be poorly outlined and only vaguely distinguishable or on account of their co existence in the same cell

The authors believe that the common origin and the normally close ontogenetic contiguity of both cells and still more the derivation of myeloblasts towards monocytes (as observed by them in other hemopathies) might justify the co existence of elements belonging to both types as well as the presence of apparently mixed or hybrid cells in this variety of acute leukemia which is sometimes called myeloblastic promonocytic

The neoplastic character assigned to leukemic cells whose existence is supposed by logical analogy on account of immaturity active reproduction and constant differentiation of the elements producing normal blood cells as well as their frequent or continuous exposition to the irritative action of toxoinfectious chemical or physical agents does not exclude of course an etiology a virus nor the participation in its origin of endocrine especially cortico adrenal troubles nor the exhaustion of the principle of maturation of the myelogenous or lymphogenous cell myelokentric and lymphokentric acids respectively (theory of Miller)

Differences are established clinical cytologic and pathogenetic between chronic and acute leukemias without excluding a common cause On the contrary the human organism seems to react in different ways sometimes only temporarily to the same causal factor

In the chronic forms this is shown by a homoplastic hyper regenerative hyperplasia a kind of permanent irritation of the myelo and lymphopoietic parenchyma s according to each type of leukemia similar to benign tumors of other organs although more diffuse as corresponds to a system with synergetic reactions towards the cause as yet unknown of this illness As a benign tumor it may degenerate and it frequently does so in cases of chronic myelogenous leukemia to end in an acute final and incurable phase as displastic unregenerative (non maturing) hyperplasia similar to the original acute leukemia of clinical as well as cytologic neoplasic aspect

The authors believe that the cytomorphology of leukemic cells reinforces other histopathological clinical and experimental arguments that support the theory of the neoplasic nature of this illness a theory which however is not as yet definitely proved

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III 2

Treatment of Acute Leukemia

JOSEPH H BURCHENAL*

TWO GENERAL types of agents are available at the present time for the treatment of the acute leukemias the antimetabolites[1, 2] and the hormones[3, 4] In neither group has an agent yet been discovered which will produce a cure but in both are compounds which have definitely been useful in achieving an increased survival time in patients particularly children with acute leukemia The conclusions to be presented today are based on 209 cases of acute leukemia treated by the Chemotherapy Service of Memorial Hospital since 1948 and 150 cases treated by us and by the Medical Service before that time

The vitamin folic acid (pteroylglutamic acid PGA) or its biologically more active conversion product citrovorum factor (5 formyl 5,6 7 8 tetrahydro pteroylglutamic acid) is necessary for the proper growth and maturation of the erythroid and myeloid cells of the marrow and also for the growth of leukemic cells PGA and CF take part in many biochemical reactions among others the incorporation of formate into the 2 carbon moiety of the nucleic acid purines[5] At the present time all the antimetabolites which are effective in the acute leukemias function presumably because they are chemical antagonists of either folic acid citrovorum factor or purines By virtue of their chemical formulae which are only slightly dissimilar to the above mentioned vitamins and essential nutrients these compounds are presumably able to enter into the enzyme systems into which the vitamins ordinarily enter Once the abnormal compound is in the enzyme system however it cannot function further it blocks out the normal vitamin and so it causes a relative deficiency of this vitamin[6] Since certain leukemic cells presumably need folic acid and citrovorum factor and purines more than normal cells they are somewhat selectively damaged by this relative deficiency Examples of such antimetabolites are aminopterin (4 amino PGA) amethopterin (4 amino N^{10} methyl PGA) 2 4-diamino 5 (3 ,4 -di chlorphenyl) 6 methylpyrimidine 2 6 diaminopurine and 6 mercapto purine

Stimulation of the patient s own adrenal by means of ACTH or supplying an excess of one of the adrenal hormones such as cortisone will also cause remissions *in a high percentage of children and young adults with acute leukemia* With the antimetabolites 30–50 per cent of the children may be expected to get good clinical and hematologic remissions[2] With ACTH and cortisone 40–70 per cent of the children and perhaps 25 per cent of young adults under the age of 30 will get good remissions[4]

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The actions of ACTH and cortisone on acute leukemia seem to be about the same although ACTH acts by stimulating the patients own adrenal to produce steroid hormones whereas the administration of *cortisone provides the patient* with an excess of one adrenal hormone

Aqueous ACTH is generally given intramuscularly in divided doses four times daily The average daily dose for a child of five would be 60 to 100 mg, for an adult 100 to 200 mg ACTH gel however can be given once daily in the same dosage *A considerably smaller dose 20 to 30 mg daily, suffices when the aqueous* drug is administered by constant intravenous drip over a period of 12 to 24 hours each day Cortisone can be given intramuscularly either in divided doses or once daily By mouth however, the absorption is more rapid and the dosage should be given four times daily The usual dose of cortisone, orally or intramuscularly for a child is 100 to 200 mg daily and for an adult 100 to 400 mg

The prolonged administration of either of these two substances frequently is accompanied by certain undesirable side effects Cushings facies excess sodium retention and consequent edema loss of weight in adults due to the catabolic effects of the drug metabolic alkalosis and hypokalemia are either not serious or are preventable by careful management Severe hypertension sometimes accompanied by hypertensive encephalopathy in children and psychic changes ranging from mild anxiety to true psychoses in adults are contraindications to further dosage The appearance of overwhelming infection in patients who have received prolonged treatment with ACTH or cortisone is not uncommon This should be met with massive antibiotic therapy immediately but whether the steroids should be discontinued entirely or increased to meet the stress of the infection is a moot point at the present time

The steroids cause rapid temporary remissions in a high percentage of children and some young adults with acute leukemia These remissions however are of short duration 1 to 12 weeks in our experience and are repeatable usually only once in children and not at all in adults In patients over the age of 30 we have seen no real remissions although subjective improvement has been noted

In discussing the antimetabolites there are several bits of experimental evidence which should be cited There has been much investigation of the effects of the antimetabolites on various strains of transplanted leukemia in mice In certain strains they are without effect but in most lines definite prolongation of survival is achieved varying from a 50 per cent increase in some to an indefinite survival free of disease in others[7-11] Amethopterin seems to be the most potent of this group 2 6 Diaminopurine has also been shown to increase the survival time of some lines[12] By a somewhat different technique amethopterin diaminopurine and the diaminopyrimidines[13, 14] have been shown to have definite leukotoxic effects in advanced mouse leukemia 6 Mercaptopurine a compound synthesized by Hitchings et al[15, 16] and shown to possess definite antitumor activity against Sarcoma 180 in mice by Clark et al[17] also shows antileukemic activity by this leukotoxic technique as well as an increase in survival in certain strains of mouse leukemia studied by the survival time technique[18-20] This last compound is at present undergoing clinical evaluation in the treatment of leukemia

and metastatic cancer but the results are too preliminary to discuss at the present time

Since as little as 5 to 100 mγ of amethopterin per ml of serum can be easily detected by a simple plate assay method with *S faecalis* studies of serum and tissue levels can be made easily [21] In the normal individual the ingestion of 5 mg of amethopterin by mouth on an empty stomach causes a rapid rise in blood level to a height of approximately 100 mγ/ml in the serum at 30 to 60 minutes with a rapid falling off of this level in the next 2 hours Approximately 40 to 60 per cent is excreted in the urine in 24 hours When given to an individual after a meal however the absorption is slower and the excretion occurs less rapidly so that detectable levels are present in the serum 6 to 8 hours after ingestion In the presence of impaired renal function the elevation of the serum amethopterin level is greater and more prolonged

The serum levels and excretion of both citrovorum factor and amethopterin were studied in leukemic and non leukemic patients by a technique similar to the intravenous glucose tolerance test This technique calls for the intravenous administration of 0 05 mg/kg of either CF or amethopterin and the removal of blood samples from the other arm at 5 10, 15 30 60 and 120 minutes there after Murphy et al have demonstrated that citrovorum factor is present in the serum at an appreciable level (10 to 20 mγ/ml) at zero minutes rises to high levels immediately after the injection and falls rapidly again to the same base line level within 2 hours 3 to 32 per cent is excreted in the urine in 24 hours mostly in the first 6 hours There do not appear to be significant differences between leukemic and non leukemic patients

Similar studies by Ellison et al [23] have shown that amethopterin on the other hand is not present in the serum at zero minutes but rises immediately following intravenous administration and rapidly returns to the zero level within a matter of 6 hours

Studies done at the Sloan Kettering Institute in mice by Fountain et al [24] have shown that after injection of amethopterin although the serum levels rapidly fall in a few hours to almost zero there is considerable retention of an amethopterin like substance in the livers and kidneys of normal mice The concentration of an amethopterin like substance in these organs continues unchanged for a period of 21 days after injection and seems within the limits of the dosage used in this experiment (0 5 to 20 0 mg/kg) to be relatively independent of the amount of amethopterin injected [5] Chromatographic and bioautographic studies on this substance lead us to believe that it is actually amethopterin rather than the deaminated derivative N[10] methylpteroylglutamic acid [25]

Further studies on patients to determine the fate of the amethopterin are in progress, and the citrovorum factor and amethopterin content of leukemic white cells following an intravenous injection of these substances are being investigated at the present time

It is also worth mentioning the relationship between citrovorum factor (leucovorin) and amethopterin The toxicity in mice of massive doses of amethopterin may be prevented by the simultaneous or prior administration of relatively small

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detected[31] Thus therapy can be reinstituted and the marrow returned to normal before any deterioration in the patient's clinical condition occurs

Another antimetabolite which has been studied in the therapy of acute leukemia is 2 4-diamino-5 (3′,4′-dichlorphenyl) 6 methylpyrimidine otherwise known as SK 5265 one of a series of compounds with antimalarial activity synthesized by Hitchings et al[2] The antitumor activity of this compound in mice was first noted by Clark et al [33] and subsequent studies showed it to be also active against other mouse and rat tumors[34] and against mouse leukemia [14]

In some bacteria[35] and experimental animals [34] its toxic effects can be prevented to a limited extent by CF but not by PGA and in the marrows of dogs[36] or patients[37] treated with this compound the appearance of megaloblasts is frequent It would thus appear that SK 5265 probably acts on the same metabolic pathway as amethopterin but at a somewhat higher locus When given in a dosage of 2 5 to 5 0 mg orally daily to children with previously untreated acute leukemia remissions have been produced in 4 out of 14 patients In 14 patients whose disease had become resistant to amethopterin however no beneficial results were obtained This drug did not appear to accomplish anything that amethopterin could not do and since its administration was occasionally accompanied by marked evidence of toxicity such as rash oral and gastro-intestinal ulcerations and hemorrhagic diarrhea it was not considered to be a practical agent in the treatment of acute leukemia [37]

Resistance

No matter how successful the original chemotherapeutic attack in a given case of acute leukemia the disease finally develops resistance to therapy and goes on unchecked to a fatal outcome It has been demonstrated that this development of resistance by cells of transplanted mouse leukemia is very similar to the drug fastness acquired by bacteria [39 40] Co-operative studies by the Sloan Kettering and Lederle groups have shown that a strain of *S faecalis* made resistant to amethopterin[41] was much more efficient than the parent strain in converting PGA to CF [4] Similar studies with cells of amethopterin sensitive and amethopterin resistant strains of mouse leukemia are underway in an attempt to elucidate the mechanism whereby resistance to therapy develops

Although several remissions are usually achieved in a given patient with the antimetabolites and occasionally with the steroids eventually the disease becomes resistant and the same drug even if pushed to toxicity fails to produce a remission It is fortunate however that there does not appear to be a cross resistance between the folic acid antagonists and the steroids [43] Patients not responding initially to amethopterin may respond to the steroids and those who responded at first and who have later developed resistance to amethopterin will still respond to steroids and vice versa In some cases after a course of ACTH or cortisone there is even a temporary return to amethopterin sensitivity Although many investigators[44 45] have tried the simultaneous administration of steroids and antimetabolites it is our impression that this has very few advantages in the average case over the method of alternation of therapy When both

doses of citrovorum factor and thus 30 mg/kg of citrovorum factor will protect a mouse against the simultaneous administration of 100 mg/kg of amethopterin when both are given daily for 5 consecutive days[26] This is roughly 50 times the LD_{50} dose of amethopterin[27] It is *important to note however, that the antileukemic effect of amethopterin in mice with transplanted leukemia can also be prevented by citrovorum factor*[5] and thus there would not seem to be any advantage in treating a patient simultaneously with citrovorum factor and amethopterin in an attempt to avoid the toxic effects of the antimetabolite A case in point is that of a 4 year old child who developed toxicity to amethopterin after being given 2 5 mg daily for 8 doses but whose dosage of amethopterin was continued and gradually increased in the presence of small doses of citrovorum factor until finally for a period of 3 weeks daily doses of 45 to 60 mg of amethopterin were given without toxicity when these doses were accompanied by the intramuscular administration of 3 mg daily of synthetic citrovorum factor (leucovorin)[29] It is also to be noted however that during this period of time when the child was not in toxicity no beneficial effects on the leukemic cells in *the marrow were noted from these very massive doses of amethopterin In the* patient with severe toxicity due to the folic acid antagonist it has been reported that massive doses of CF will reverse these toxic manifestations[30]

The therapeutic dosage of amethopterin generally employed in our clinic is 2 5 mg daily for a five year old child[31] A relatively comparable dose of aminopterin would be 0 25 to 0 5 mg daily Both of these compounds can be given orally and absorption has been shown to be equally rapid orally or intramuscularly

Ulcerations of the buccal mucosa are usually the first sign of toxicity to amethopterin *These are frequently painful and are usually called to the attention* of the doctor by the patient himself The ulcerations are covered with yellowish white exudate and are surrounded by a reddish areola In themselves they are not dangerous but are often precursors of ulcerations farther down in the gastrointestinal tract which may be dangerous in a patient who already has a bleeding diathesis Other signs of toxicity from this drug are anorexia loss of hair temporary interference with beard growth leukopenia and thrombopenia Our standard plan of treatment[31] in children with acute leukemia is to administer amethopterin orally at 2 5 mg *daily for at least 3 weeks until such time as a* remission occurs as evidenced by the return of the marrow to an essentially normal morphology and function or until definite signs of toxicity appear If toxicity does occur treatment is temporarily discontinued for a period of 7 to 10 days and then started again at a somewhat lower dosage when the signs subside Treatment should then be continued until a remission occurs and in the absence of a remission if the patient is in a reasonably good condition treatment can be continued for 2 months or more Dosage should be increased to the point *of mild toxicity in hopes of attaining a remission* When once a remission is achieved the patient may be continued on a maintenance dosage of the drug or therapy may be discontinued When the intermittent schedule is used however aspiration of the sternal marrow *should be done every 2 weeks during the* remission as it is only by this technique that the first signs of relapse can be

toxicidad técnica de terapia y los resultados que deben esperarse de los distintos tipos de terapia antimetabólica serán discutidos

La estimulación de la suprarrenal del paciente por medio del ACTH o suministrando un exceso de una de las hormonas adrenales como la cortisona causará remisiones en un alto porcentaje de niños y jóvenes adultos con leucemias agudas

La dosificación vía de administración y efectos fisiológicos de estos compuestos serán discutidos

Con los antimetabolicos en el 30-40% de los niños se puede esperar una buena remisión clínica y hematológica

Con ACTH y cortisona 40-70% de los niños tendrán una buena remisión y quizá el 25% de jóvenes adultos menores de 30 años Creemos que hay ciertas indicaciones para el uso de estas drogas Si el paciente es un niño con una cifra de blancos menor de 50 000 y parece estar en condiciones de vivir por 2 o 3 semanas sin terapéutica le administramos ametopterina porque creemos que en los pacientes en los cuales se pueden obtener beneficios con los antimetabolicos las remisiones a continuación del tratamiento con esta droga duran más tiempo y pueden repetirse más que con las hormonas

Si el paciente es un joven adulto o un niño con una cifra de blancos de más de 50 000 o un niño cuya enfermedad es aguda y no parece tener una supervivencia suficientemente larga para darle a la ametopterina oportunidad de demostrar sus efectos benéficos comenzamos entonces un tratamiento con ACTH o cortisona

Si la emergencia es extrema el uso de ACTH endovenoso parece tener un efecto más rápido Entre las hormonas bajo condiciones ordinarias sin embargo la cortisona por boca es la droga de elección por su fácil administración En aquellos niños que responden a la ametopterina esta droga es la preferida Cuando la leucemia se hace resistente a esta droga puede ser tratada con ACTH o cortisona lográndose más remisiones pero después de cada remisión con ACTH o cortisona se prueba nuevamente ametopterina cuando ocurre otra recaída

Una serie de 155 casos de leucemia aguda fueron tratados en el Memorial Hospital de Marzo de 1948 a Marzo de 1951 con los antagonistas del ácido fólico A algunos enfermos se les dió además ACTH y cortisona 40 vivían 12 meses después de la iniciación de la enfermedad contrastando con 2 en 150 de una serie de leucemias agudas no tratadas con antagonistas del ácido folico o cortisona y ACTH previamente comunicadas en el Memorial Hospital por Southam *et al* Seis de nuestros pacientes sobrevivían 24 meses después de la iniciación de su enfermedad y uno aun vive y está en buenas condiciones 42 meses después del comienzo de la enfermedad Nosotros creemos que con el uso de los antagonistas del ácido fólico y de las hormonas un aumento definido de la supervivencia puede ser alcanzado en muchos niños con leucemia aguda

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drugs are given simultaneously it is felt that *resistance to both will develop* and the therapist will be left without effective agents sooner than if one drug at a time were used

We feel there are certain indications for the use of these various drugs If the patient is a child below the age of 10 years with a total leukocyte count under 50 000 and appears to be in such shape that he will live for 2 or 3 weeks without therapy we then start him on amethopterin because we feel that in the patients in whom benefit can be achieved by the antimetabolites remissions following these drugs last longer and are more repeatable than with the hormones If the patient is a young adult or a child with a white count over 50 000 or a child who is very acutely ill, and does not appear likely to survive long enough to give amethopterin a chance to demonstrate its beneficial effect we then start the patient on ACTH or cortisone If there is an extreme emergency the use of ACTH intravenously seems to have the most prompt effect Among the hormones under *ordinary conditions however, cortisone by mouth is the drug of choice* because of the ease of administration *If a remission is achieved by these drugs* therapy is stopped temporarily and reinstituted with amethopterin when a relapse *occurs Thus in most cases* except when special circumstances exist we feel that amethopterin should be the main reliance of the chemotherapist in the acute leukemias of childhood with ACTH and cortisone being reserved for those special instances where amethopterin has either failed or would not be expected to work rapidly enough

A series of 155 cases of acute leukemia were treated at Memorial Hospital from March 1948 to March 1951 with the folic acid antagonists Some of these were also given ACTH and cortisone in addition Forty were alive 12 months after the start of their disease contrasted with 2 out of 150 from a series of acute leukemias not treated with folic acid antagonists or cortisone and ACTH previously reported from Memorial Hospital by Southam et al [46] Seven of our patients were surviving 24 months after the start of their disease and one is still alive and well 48 months after the start of the disease We feel that by the use of the folic acid antagonists and the hormones a definite increase in survival time can be achieved in many children with acute leukemia

Tratamiento de la Leucemia Acuda

Se disponen actualmente de dos grupos de agentes generales para el tratamiento de las leucemias agudas

Ninguno de éstos produce la curacion pero ambos han sido definitivamente utiles alargando el tiempo de supervivencia de los pacientes particularmente en los ninos afecta los por leucemia

La vitamina ácido folico (ácido pteroylglutámico) es indispensable para el debido crecimiento y maduracion de las células eritroides y mieloides de la médula y también para el crecimiento *de las células leucémicas Pareceria que en* ciertas leucemias agudas las células leucémicas necesitan ácido folico o el biologicamente más activo ácido folinico (ácido 5 formil 5 6 7 8 tetrahidropteroilglutámico) en mayor cantidad que las células normales de la médula

Al administrar a estos pacientes un antagonico de esta vitamina como la aminopterina ametopterina o diamino diclorofenilpirimidina sus células leucémicas son específicamente danadas por la relativa deficiencia de ácido folínico con ellos lograda La farmocologia

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III 3

The Treatment of Acute Leukemia in Children by Folic Acid Antagonists, ACTH and Cortisone A Summary of Five Years' Experience

SIDNEY FARBER, RUTH APPLETON, VIRGINIA DOWNING, FELIX HEALD, JAMES KING and RUDOLPH FOCH*

AT THE First Congress of the International Society of Hematology in 1948 we had the opportunity to present an extension of our first report made earlier that year concerning the production of remissions in somewhat more than 50% of children with acute leukemia treated for three weeks or longer with either aminopterin amethopterin or amino an fol The limitation of this form of therapy was described and the problems to be solved before further progress was possible were defined *The demonstration that a family of biologi* cally active chemical compounds the folic acid antagonists possessed a carcinolytic effect not only on the leukemic process but also on other unrelated forms of cancer such as the neuroblastoma served as a stimulus to a search for other compounds equally or more effective as carcinolytic agents and less toxic to the normal tissues of the body

In accepting the gracious invitation to present a paper today we would like to pay tribute to the large number of scientists in several disciplines whose work made this clinical *advance possible We should like to express our gratitude specifically to the late Dr Y* Subba Row and to his successor Dr James Williams and their colleagues in the Lederle Research Laboratories of the American Cyanamid Company whose research contributions are interwoven throughout the fabric of our own studies It is pleasant to acknowledge the constant support both clinically and scientifically given by Dr James Ruegsegger Medical Director of the Research Division of the Lederle Laboratories who is kind enough to present this summary of our experience of the past five years in our absence today

General Treatment of the Patient with Acute Leukemia

One of the most important consequences of the first successful albeit temporary chemotherapy of acute leukemia was the increase in interest in the patient with incurable cancer All patients with acute leukemia receive what we have termed *total care* This term is employed to describe the finest medical and surgical treatment available today for the comfort well being and increased survival of the patient with incurable disease It includes the use of blood transfusions fluids designed to restore the acid base equilibrium and the proper hydration of the tissues antimicrobial agents for the prevention and treatment of complicat

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ing infectious disease local or generalized roentgen therapy and surgical technics even for temporary relief of symptoms resulting from obstruction or pressure and finally attention to the happiness the mental state and the economic problems of the patient and his family As part of this total care there is introduced the anti tumor agent whether chemical or hormonal in nature It is clear that such total care alters the life history of the disorder The accumulation of data however from a large number of children with acute leukemia who required nothing beyond good general care and the rapidity of action of the folic acid antagonists in so many instances make possible objective conclusions concerning the carcinolytic action of the folic acid antagonists in acute leukemia In a large number of patients however the total care made possible the survival of the patient until folic acid antagonist therapy could become effective In many instances too the continued survival at times of resistance to the action of the folic acid antagonists was dependent upon the general care

Carcinolytic Agents Employed

Studies have been made of the effect of a series of folic acid antagonists on acute leukemia in children Two weak folic acid antagonists An fol A and Met fol B were employed in our original studies beginning in March 1947 Dramatic remissions were not achieved but definite carcinolytic action was observed recognizable by microscopic studies both of bone marrow and of tissues Remissions have been obtained following treatment with aminopterin amethopterin amino an fol aminopterin adenopterin aminoteropterin and in one instance after dichloraminopterin Of these the most satisfactory were found to be aminopterin amethopterin and aminoteropterin although it is possible that further use of the other related compounds named above might have demonstrated equally good results Since there appeared to be no advantage of aminoteropterin over aminopterin and amethopterin we have dropped aminoteropterin except for purely experimental purposes and used either aminopterin or amethopterin for routine treatment of the child with acute leukemia

A group of compounds related to folic acid in structure but without the amino group in the 4 position such as 9 10 dimethylpteroyl glutamic acid proved to have no effect in acute leukemia in children although the survival time of the mouse with transplantable leukemia was definitely increased So far no folic acid antagonist of the aminopterin series without the amino in the 4 position has had carcinolytic action in man All effective compounds have a toxic level definitely higher but quite close to the therapeutic level

ACTH and Cortisone

The early observation of Heilman and Kendall of a temporary regression of a lymphoid tumor in mice with the administration of cortisone the protection against transplanted leukemia in rats by the administration of adrenal cortical hormones and ACTH described by Murphy and Sturm and the demonstration of the depressing effect of ACTH on circulating lymphocytes by Dougherty and White made logical the trial of ACTH and cortisone on acute leukemia in man Our experience with the production of remissions in children with acute leukemia

following treatment with ACTH beginning in 1948 was in agreement with results following ACTH therapy in a number of other clinics Similar effects were produced by the use of cortisone

There has been some variation in general experience and a few of these may be cited Mila Pierce reported the production of an overall remission rate of 46% in 26 children with acute leukemia with repeated remissions in 6 patients One lasted for six months and one for 12 months on ACTH alone Wintrobe was able to report remissions in more than 50% of a small series of children with a duration of one to two months In a series of 14 patients described by Franklin treated with ACTH and cortisone a remission rate of 54 5% was obtained, with 16 complete and 8 partial remissions The average remission was 48 5 days with a variation from 16 to 135 days Snell and his colleagues in Toronto described data from 16 patients with acute leukemia Complete but temporary remissions occurred in a large proportion Most patients in whom remissions were produced were almost completely refractory to re treatment Our own experience led us to use ACTH and corti one as adjuncts to folic acid antagonist therapy when required under conditions which will be considered below

Dihydrotriazines

Clinical observations have been made on a small series of children with acute leukemia of the effect of two dihydrotriazines—p chlorophenyl 1 2 dihydro 2,2 dimethyl s triazine (our name D 20) and the dichlor analogue (D 54) These representatives of a new family of chemical compounds synthesized by Modest in our laboratories proved to have anti folic anti citrovorum factor anti niacin and anti malarial activity In mice there was a definite anti leukemic effect Clinical studies still in preliminary form do not yet permit conclusions

Response to Therapy—Illustrative Examples

The response of children with acute leukemia to treatment may be illustrated by the courses of three patients The first of these (N B) may be regarded as an average of typical experience characteristic of a large number of our patients who responded for a time Her course ran from August 1950 to November 1951 when she died because of overwhelming sepsis

The most prolonged remission ascribable completely to folic acid antagonist therapy was observed in a boy (M S) now 5 years 10 months of age who has been in complete hematological and clinical remission for 35 months He has been treated solely with a folic acid antagonist which he has received daily and continuously since September 1949

The patient with the longest survival following proof of existence of acute leukemia is a girl (M M) now 9 8/12 years of age who has survived for 42 months She is now in hematological relapse after having had mixed forms of therapy but is in good clinical condition Her initial remission was obtained with folic acid antagonist therapy Her course illustrates the application of total care repeatedly when specific therapy was temporarily of no value

Response to Folic Acid Antagonist Therapy

In 1951 at the Second Folic Acid Antagonist Conference in Boston the experience in a number of cities was gathered The significant improvement rate of some 425 children including 190 under our care in New York Cleveland Chicago Ann Arbor Rochester Minnesota Philadelphia and Boston to treatment with folic acid antagonists for 14 days or more averaged 68 5% which is

very close to the figure of 64% for 245 children treated up to February 1952 The variation in percentage from city to city may be explained in part by the small series in some of the clinics whose figures might go up or down had larger numbers of children been treated A few series of adult patients with acute leukemia treated with folic acid antagonists show an improvement rate that varies between 10 and 15%

Response to Folic Acid Antagonist Therapy in Patients with Acute Leukemia Treated More than 21 Days (January 1 1948 to February 1 1952)

During this period 243 patients were treated for three weeks or longer Their response may be described as follows

(1) 195 patients were treated initially with folic acid antagonists Of these 103 or 52 8% showed an important degree of improvement without infection and 18 or 9 2% showed important improvement in the presence of infection, making a total of 121 or 62% who showed a significant degree of improvement 25 2% of those that did respond showed complete clinical and hematological remission The remainder 74 or 38% failed to respond

2 Of the 243 patients treated 3 weeks or longer, 48 or 19 8% had folic acid antagonists and ACTH or cortisone as the initial treatment 58 3% showed an important degree of improvement without infection and 4 or 8 4% improved in the presence of infection making a total of 33/48 or 66 7% with improvement

In 11 of 49 children or 25 8% with acute leukemia who had complete remissions due to folic acid antagonists alone the relapse which occurred later was changed to remission by the use of cortisone or ACTH In 27 who had marked improvement, 55 5% initially treated with folic acid antagonists with subsequent relapse 15 patients were once more improved by the use of ACTH or cortisone with complete remissions in 6 (2 of these had definite infection) 74 children did not respond to initial treatment with folic acid antagonists Of these 9 were markedly improved subsequently by ACTH or cortisone Infection played an important role in 2 of these 9 Those who did not respond to ACTH or cortisone occasionally went into remission on subsequent folic acid antagonist therapy

Survival Rate

Following our original description concerning the effect of aminopterin on acute leukemia considerable discussion was raised concerning the variation in the survival period in untreated patients Our own conclusions concerning the effect of specific therapy were based upon the records of 300 children with acute leukemia seen during the previous 25 years in one institution The most recent review of survival in the leukemias of childhood has been made by Tivey He analyzed the published papers from 1927 to 1950 listing a total of 428 instances of acute leukemia He pointed out that the *average* survival of patients is a misleading indication of both the most probable time of survival and the midpoint of the series He suggested the application of a simple statistical technic of summarizing survival on log probability paper From these studies he concluded that 50% of the children with acute leukemia given supportive therapy and not antibiotics will die within a period of approximately 4 months after the onset of

the first definitive symptoms, about 10% will live as long as 11 months It may be expected the middle two thirds will survive from approximately 2 to 8 months

The following survival rates have been derived at from our experience on 337 patients most of whom were children with acute leukemia treated in the period from January 1 1948 to August 15 1952 These patients are unselected and include all who died at any time after coming under observation including the first three weeks of treatment 276 of these patients with acute leukemia treated for 21 days or longer form a special group for consideration The results may be summarized

(1) 50% of the 428 patients reported by Tivey survived for 4 months 50% of 337 unselected patients in our experience were living at 7 months while 50% of 276 treated for more than 21 days were living at 8 3 months after the onset

(2) The figures in the three groups for the survival of 10% of the patients are 11 months from Tivey s tabulation, and 16 and 18 months in the two groups in our experience A more detailed summary follows

On August 15 1952 71 (21 2%) of the 337 unselected patients with acute leukemia who had been treated with folic acid antagonists had survived for 12 months 44 or 13% have survived 15 months, 29 or 8 6% 18 months 18 or 5 4% 21 months and 12 or 3 6% for two years 5 or 1 5% survived for 32 months

The group of patients who have responded best in our experience were the 92 who received folic acid antagonist therapy initially and ACTH or cortisone after a subsequent relapse This is a selected group since it presupposes that the patient lived long enough to receive both forms of therapy However it represents survival times of those for whom the drugs are most helpful In this group 42, or 45 6% survived 12 months 30 or 32 6% 15 months 20 or 21 7% 18 months 14 or 15 2% 21 months and 9 or 9 8% have survived for two years One patient is alive 42 months after the onset

These survival times are significantly greater than those in acute leukemia prior to the use of folic acid antagonists and greater than those patients who did not respond to folic acid antagonist therapy

Toxicity of the Folic Acid Antagonists

The important pathologic lesions produced by toxic levels of the folic acid antagonists originally discouraged many clinical investigators It was emphasized in our early studies that it was possible to administer any one of the folic acid antagonists in a dose computed for the individual patient without causing stomatitis gastrointestinal changes or aplastic anemia In 65% of the patients in whom remissions have been obtained in our experience no evidence of toxicity was observed prior to the onset of the remission The conclusion appears permissible that the toxic effects and the carcinolytic effects may be separated The citrovorum factor (leucovorin) in appropriate amounts will nullify the effects of the folic acid antagonists It is of great theoretical importance but its role in the treatment of acute leukemia has not been clarified

Resistance

Approximately one third of all patients with acute leukemia are resistant to the effects of folic acid antagonists from the very beginning It is possible that we are concerned here with a biologically and chemically different problem in these two great classes of patients with acute leukemia Quite different in all likelihood from this *initial* resistance is that which is manifested after the patient has once responded satisfactorily as shown by clinical and hematological studies to the folic acid antagonists Eventually most patients and perhaps all will manifest evidences of this form of *acquired* resistance to the folic acid antagonist employed The large amount of research activity initiated to explain and over come this phenomenon has not yielded a satisfactory solution When that prob lem is solved the usefulness of folic acid antagonists in acute leukemia may well be compared to that of insulin in the treatment of diabetes

Suggested Treatment Regime

In general if a patient is not critically ill when the diagnosis of acute leukemia is made folic acid antagonists are employed alone to be followed by ACTH or cortisone in time of relapse or initial resistance Should the patient be critically ill and should there be obvious necessity for rapid improvement if the patient is to survive ACTH or cortisone is employed initially to be accompanied by or followed in as short a period of time as possible by folic acid antagonist therapy ACTH or cortisone is employed initially also when there are large lymphosarco matous masses as for example in the mediastinum in the child with acute leukemia and also in adolescent children In these combinations cortisone and folic acid antagonist therapy has given the best results In several patients ACTH has been responsible for remissions up to five weeks after failure was encountered following originally successful remissions produced by the action of folic acid antagonists and cortisone The poorest responses to treatment by either folic acid antagonist or ACTH and cortisone were observed in adults in adolescent children beyond puberty in patients with large lymphosarcomatous masses in the mediastinum or elsewhere and in children who on admission had extremely high white blood cell counts Only 10% of children with high white counts on admission have developed complete remissions in our experience

Conclusions

The treatment of acute leukemia includes total care and the application of the special forms of treatment—folic acid antagonists (aminopterin amethopterin) and ACTH or cortisone The folic acid antagonists produce longer and more repeated remissions than is the case with the ACTH or cortisone alone In a large series of patients the survival time has been prolonged appreciably because of treatment with folic acid antagonists and ACTH or cortisone There is a small number of survivors beyond two years one child is still alive 42 months after onset of her acute leukemia The use of the folic acid antagonists notably aminopterin and amethopterin has reached the stage of routine value as part of the total care of the child with acute leukemia This statement is made even though the phenomenon of either initial or acquired resistance of the leukemic process to the folic acid antagonist precludes the possibility of either permanent

cure or indefinite survival today For it is the eventual resistance of the leukemic cell to either the folic acid antagonist or the hormone employed that constitutes the important barrier separating increased survival and temporary remissions from the long term improvement of the type achieved in pernicious anemia and diabetes by modern forms of therapy The solution of this problem therefore, is a prime requisite for further progress in this direction The increase in survival of so many children with acute leukemia attributable to hemotherapy has all the greater value in view of the rapid progress in the solution of biological and chemical problems of importance to the patient with acute leukemia

The term 'temporary remission' in response to aminopterin employed in our initial observations five years ago remains unaltered in describing the effect of folic acid antagonists or any other form of therapy in acute leukemia today Accumulating experience during these years however, has established the role of these compounds and of ACTH and cortisone in the routine care of the child with acute leukemia with a goal of prolonging the life and increasing the well being of the child with a disorder which is still incurable Whether the next step comes in the form of a solution to the problem of resistance of the leukemic cell to the treatment employed or in the form of another chemical compound, a hormone or a filterable virus great experience is at hand for its rapid application to the child with acute leukemia and for the critical evaluation of its efficacy

Tratamiento de la Leucemia Aguda en Niños por Antagonistas del Acido Folico ACTH y Cortisona Un Resumen de 5 Años de Experiencia

El tratamiento de la leucemia aguda abarca los cuidados generales y la aplicacion de formas especiales de tratamiento antagonistas del ácido fólico (aminopterina ametopterina) y ACTH o cortisona Los antagonistas del fólico producen remisiones más largas y repetidas que las que produce la ACTH o la cortisona solas En muchos pacientes la supervivencia se ha prolongado notablemente mediante esos tratamientos

Existe un pequeño numero de supervivientes que han llegado a los dos años y un niño vive todavía 42 meses después de la iniciación de la enfermedad El empleo de los antagonistas del ácido folico particularmente la aminopterina y la ametopterina ha llegado ya a la fase de rutina como parte del tratamiento de los ninos con leucemia aguda Se establece este juicio a pesar de que el fenomeno de la resistencia inicial o adquirida de los procesos leucemicos a los antagonistas del ácido fólico excluye la posibilidad de un tratamiento permanente o de una supervivencia indefinida Esa posible resistencia de la célula leucémica tanto a los antagonistas como a las hormonas constituye la importante barrera que separa el aumento de la supervivencia y las remisiones temporales de la mejoría a largo plazo del tipo obtenido en la anemia perniciosa o en la diabetes por las modernas formas de terapia La solución de ese problema es el requisito esencial para los progresos ulteriores en esa direccion El aumento de la supervivencia en muchos ninos leucémicos atribuible a la hemoterapia tiene también gran valor en vista de los rápidos progresos en la solución de importantes problemas biologicos y químicos referentes a estos pacientes

El término remisión temporal empleado en las observaciones iniciales del autor hace 5 anos tiene igual significacion actualmente Sin embargo la experiencia acumulada en estos anos ha establecido el papel de estos compuestos y de la ACTH y cortisona en el tratamiento de rutina de los niños con leucemia aguda con el fin de prolongar la vida y mejorar el bienestar del niño afecto de un proceso aun incurable Estos estudios nos han dado gran experiencia para poder ampliar y valorar otros métodos que puedan contribuir a la solución bien sea resolviendo el problema de la resistencia de las células leucémicas a la sustancias hasta ahora empleadas bien sea en la forma de encontrar otros compuestos químicos u hormonas o por la vía de los virus filtrables

III 4

The Physiopathology of Treated Acute Leukemias

J BERNARD and G MATHE*

THE THERAPEUTIC measures used in acute leukemias are of two types (1) those that occasionally produce remissions (colchicin urethane nitrogen mustards radioactive phosphorus estrogens) (2) those that are capable of producing frequent remissions (exchange transfusions folic acid antagonists cortisone and ACTH)

The exchange transfusions or the repeated transfusions given in special conditions (such as leukemic donors in remission or donors treated with cortisone) may give 20 to 25 per cent remissions which would lead one to think that there would be anti leukemic substances in the blood of these individuals

The use of folic acid antagonists permits us to study the specific action of each of them their way of introduction their dosage the duration of treatment the use of maintenance doses their association with transfusions the use of antidotes (citrovorum factor folic acid)

The use of hormones (cortisone ACTH) permits us to vary the conditions mentioned for a comparative study

With the help of folic acid antagonists or with hormones up to 30% complete remissions may be obtained a figure which can be higher when both medications are administered simultaneously

In performing such studies the clinical manifestations have been recorded and hematologic controls have been performed both in peripheral blood and bone marrow obtaining the results of various medullar sectors (sternum iliac crests tibia posterior processes of vertebrae) To the classic smears stained with May Grunwald Giemsa the examination with phase microscopy has been added

The chemical investigations have included the study of the metabolism of folic acid citrovorum factor and folic acid antagonists and have also determined the glutathionemia both in treated and untreated leukemias It has been noted that in the untreated cases the relationship of total glutathion and the number of red cells on one hand and the relationship between the oxidized glutathion and the total glutathion on the other hand were elevated in both cases With the use of immuno chemical methods the behavior of serum globulins and the urinary steroid elimination were studied Our attention has also been directed towards radioactive tracers and a detailed study has been made of the existing anatomical and endocrine glands alterations

Although it is not yet possible to completely cover the physiopathology of the treated acute leukemias we may consider the problems that should be investigated

The different sensitivity of different cases which will depend on the age (more

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frequent remissions in children) the type of cell involved (more frequent remission in the forms with leucoblasts without granulations and absence of remission in the monocytic and reticular cell leukemias) the anatomo clinical forms (the leucosarcomas are very sensitive) and some other unknown factors

The type of remission, which underlines the necessity of defining the significance and the anatomical findings of complete remissions

The mechanism of action both of folic acid antagonists and hormones, a problem that though not yet solved leads to important theories

Finally other problems of main importance are the secondary resistance that develops in the course of treatments, independently for anti folic and hormones and the influence of infections in the treated leukemias

La Fisiopatología de las Leucemias Agudas Tratadas

Los métodos terapéuticos empleados en las leucemias agudas son de dos tipos primero aquéllos que habiendo sido empleados en pocos casos provocan raras remisiones (colchicina uretano mostazas nitrogenadas fosforo radioactivo estrógenos) segundo aquéllos que son capaces de provocar frecuentes remisiones (exsanguíneo transfusiones antagonistas del ácido folico cortisona y ACTH)

Las exsanguineotransfusiones o las transfusiones abundantes practicadas en condiciones especiales (dadores específicos tales como leucémicos en remisión o dadores tratados con cortisona) pueden provocar del 20 al 25% de remisiones lo que permite suponer que existen substancias antileucémicas en la sangre de los sujetos sanos

El empleo de antagonistas del ácido folico permite estudiar la acción específica de cada uno de ellos las vías de aplicación su posología la duración del tratamiento el empleo de dosis de mantenimiento su asociacion con transfusiones el empleo de antídotos (factor citrovorum ácido fólico)

El empleo de hormonas (cortisona ACTH) permite variar las condiciones mencionadas para un estudio comparativo

Con la ayuda de antagonistas del ácido fólico así como con hormonas se obtiene hasta 30% de remisiones completas proporción que puede ser mayor cuando se administran simultáneamente los dos medicamentos

Al realizar tales estudios se han vigilado las manifestaciones clínicas y se han practicado controles hematológicos tanto de la sangre periférica con todas las particularidades de las curvas de eritrocitos leucocitos y plaquetas como de la médula ósea obteniendo el producto de varios sectores medulares (esternon crestas ilíacas tibias apofisis espinosas)

Al clásico estudio de los frotis coloreados con May Grunwald Giemsa se ha añadido el examen con microscopio de contraste de fase

Las investigaciones químicas han abarcado el estudio del metabolismo del ácido folico factor citrovorum y de los antagonistas del ácido fólico y también han tenido por objeto la determinación de la glutationemia tanto en las leucemias tratadas como en las no tratadas habiéndose observado que en estas ultimas se eleva la relacion entre el glutatión total y el numero de globulos rojos y la relación entre el glutatión oxidado y el glutatión total También se ha investigado por los métodos inmunoquímicos el comportamiento de las globulinas séricas y se ha precisado la eliminación de los esteroides urinarios

Se ha dirigido también la atención a los marcadores radioactivos y se ha hecho un estudio detenido de las alteraciones anatómicas existentes haciendo extensiva la investigación a las glándulas endocrinas

Aunque aun no es posible abarcar en conjunto la fisiopatología de las leucemias agudas tratadas se pueden plantear los problemas más importantes que deben ser examinados

La desigual sensibilidad de los diferentes casos que depende de la edad (remisiones más frecuentes en los niños) del tipo citologico (remisiones más frecuentes en las formas a leucoblastos medios sin granos y ausencia de remisiones en las leucemias a monocitos y a

células reticulares) de la forma anátomo clínica (las leuco sarcomatosis son muy sensibles) y de factores por el momento desconocidos

El tipo de remisión, siendo necesario definir la significación y los hallazgos anatómicos de las remisiones completas

El mecanismo de acción, tanto de los antagonistas del ácido fólico como de las hormonas problema que aunque no ha sido aún resuelto se presta a importantes conjeturas

Finalmente otros problemas de particular importancia son la *resistencia secundaria* que se desarrolla en el curso de los tratamientos de un modo independiente para los antifólicos y las hormonas y *la influencia de las infecciones* en los leucémicos tratados

III 5

Eritro-Leucemia

GIOVANNI DI GUGLIELMO*

LAS ENFERMEDADES mieloproliferativas caracterizadas por la proliferación de los elementos celulares del tejido mieloide se pueden ordenar en dos clases

La *primera clase* abarca las *enfermedades mieloproliferativas puras* (es decir con proliferación electiva de uno solamente de los tres sistemas celulares del tejido mieloide) que son a saber

1) leucemia mielógena
2) eritremia
3) megacariocitemia
4) policitemia vera

La *segunda clase* abarca las *enfermedades mieloproliferativas mixtas* (es decir con proliferación de dos o incluso de los tres sistemas celulares del tejido mieloide) Las formas con *doble componente proliferante* son las siguientes

1) eritoleucemia
2) eritomegacariocitemia
3) leucomegacariocitemia

Cuando el componente proliferante es *triple* esto es con proliferación de todos los elementos celulares del tejido mieloide la enfermedad es denominada *eritroleucomegacariocitemia* o *panmielosis* o *panmielopatía*

Estas distintas formas de enfermedades mieloproliferativas pueden agruparse de acuerdo con el siguiente esquema

Enfermedades Mieloproliferativas

I *Enfermedades mieloproliferativas puras*
1) Leucemia mielógena
2) Eritremia
3) Megacariocitemia
4) Policitemia vera

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frequent remissions in children) the type of cell involved (more frequent remission in the forms with leucoblasts without *granulations and absence of remission* in the monocytic and reticular cell leukemias), the anatomo clinical forms (the leucosarcomas are very sensitive) and some other unknown factors

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lares medulares (leucocitario eritrocitario y megacariocitario) a menudo proliferan simultaneamente *en masse o como unidad* mas bien que como sistemas singulares Se ha llegado así a reconocer que la proliferación de todas las células de la médula osea cualquiera que sea el sistema al que pertenecen es primitiva y que no se debe hablar de *reacción secundaria* Para demostrar la inexactitud de este concepto de reacción secundaria voy a proyectar algunas microfotografias de un caso diagnosticado de leucemia mielógena Ciertamente hay algunos elementos celulares típicos de la leucemia mielogena es decir numerosos mieloblastos en la sangre periférica pero hay asimismo numerosos proeritroblastos y eritroblastos basófilos y ademas un gran numero no sólo de megacariocitos con función trombocitopoyética sino también de típicos magarioblastos ¿Es una leucemia mielógena con reaccion eritrémica y megacariocitica? ¿O una eritremia con reacción leucémica y megacariocítica? ¿O bien una megacariocitemia con reacción eritrémica y leucémica? Ni una ni otra cosa pero sí una eritroleucomegariocitemia por proliferación primitiva contemporanea de los tres sistemas celulares del tejido mieloide

En otros casos estos cuadros mixtos de eritroleucomegacariocitemia son interpretados como expresion de una metaplasia esplénica compensatoria consecutiva a una mielofibrosis interpretacion no siempre correcta porque con frecuencia se ha podido demostrar que la mielofibrosis es secundaria a una mieloreticulosis esto es a una proliferacion primitiva de las células reticulares de la médula osea surgida al mismo tiempo que una proliferación primitiva de las células parenquimatosas medulares Desde hace mucho tiempo es conocida la considerable proliferacion de las células reticulares de la medula osea especialmente en las eritremias agudas hasta el punto que en algunos casos no se sabe si asentar diagnóstico de eritremia con reticuloendoteliosis o de reticuloendoteliosis con eritremia Se trata en cambio de formas mixtas de *eritromielosis* + *reticulomielosis* que se pueden comprobar no tan sólo en la médula osea sino en todos los organos extramedulares (bazo hígado etc) En la evolución sucesiva la proliferación reticular de la médula osea puede llegar a ser preponderante en comparación de la parenquimatosa y producir un estado terminal de mielofibrosis con hipoplasia parenquimatosa medular Por lo tanto no se puede hablar en dichos casos de mielofibrosis pues ésta constituye una fase terminal de una mieloreticulosis primitiva

Consignada la interpretacion de los conceptos de reaccion secundaria y metaplasia compensatoria vamos ahora a fijar los caracteres que permitan asentar el diagnóstico de eritroleucemia

I *Caracteres anatomicos* — Los fundamentos anatomicos en los que apoya la enfermedad eritroleucémica son los siguientes

a) Proliferación simultanea con anomalías de maduracion de las células de la serie eritropoyética y la serie granulopoyética las investigaciones llevadas a cabo mediante cultivo *in vitro* de la médula ósea en varios casos de eritroleucemia han puesto de manifiesto anormalidades en la maduración de los elementos tanto de la serie eritropoyética como de la granulopoyética Estas *anomalias* no están relacionadas con deficiencia de factores extracelulares sino que represen

II *Enfermedades mieloproliferativas mixtas*

1) Eritroleucemia
2) Eritromegacariocitemia
3) Leucomegacariocitemia
4) Eritroleucomegacariocitemia (panmielosis o panmielopatía)

Las enfermedades mieloproliferativas así agrupadas no estan separadas entre sí *por límites demasiado rigurosos* pues cada una de ellas puede transmutarse en otra por lo cual se habla de *formas de paso* que mas exactamente deben considerarse como *fases evolutivas* de un mismo proceso mieloproliferativo

Estas enfermedades son causadas por la proliferacion de las células parenquimatosas del tejido mieloide, sin embargo hay que tener presente también la posibilidad de una *concomitante proliferacion de las células reticulares del tejido mieloide (mieloreticulosis)* la que en la sucesiva evolucion puede llegar al estado de *mielofibrosis* cuando prevalezca la proliferacion mesenquimatosa en comparación de la parenquimatosa

Mientras las enfermedades mieloproliferativas puras especialmente la leucemia mielógena cronica y la policitemia vera son muy conocidas las formas mixtas son consideradas raras y a menudo se interpretan como formas puras con reacción secundaria de otro sistema Se habla pues con frecuencia de leucemia mielogena con reacción eritrémica o megacariocítica, o bien de policitemia vera con reacción leucemoide o megacariocítica

Solo en estos ultimos anos la literatura médica internacional se ha enriquecido con numerosos casos de eritoleucemia casi todos documentados hasta en la mesa de autopsias

Se dan a continuacion las razones principales del considerable aumento de casos de enfermedades mieloproliferativas mixtas comprobados muy recientemente más extendida difusion de las biopsias medular esplénica y hepatica y sobre todo mas extendida aplicacion del tratamiento por la transfusión y los antibióticos lo cual ha alargado la vida de los pacientes afectos de hemopatías agudas Esta prolongacion de vida permite seguir la evolucion de la enfermedad a través de fases puras y fases mixtas por ejemplo en algunos casos una fase terminal de eritroleucemia es consecutiva a una fase inicial de leucemia mielogena aguda o de eritremia aguda Pero no siempre se observan estas fases evolutivas antes bien en la mayoria de los casos las leucemias agudas y las eritremias agudas empiezan como formas puras y como formas puras se terminan a pesar de la abundante administracion de antibioticos y transfusiones de sangre Asimismo muchos casos de eritroleucemia no sufren ningun cambio durante su evolución aun cuando prolongada por la administración de antibioticos y transfusiones Naturalmente no se debe equivocar la eritroleucemia (forma mixta de eritremia + leucemia) con la eritremia (forma pura electiva de la serie eritroblástica y sin alteraciones granuloblásticas) Puede haber todas las formas intermedias entre la eritremia y la leucemia mielogena pero no se debe equivocar con las formas puras

Después de las numerosas y bien documentadas observaciones de enfermedades mieloproliferativas mixtas se ha empezado a reconocer que los tres sistemas celu

En las formas crónicas de las enfermedades mieloproliferativas mixtas es más fácil observar casos con proliferación de la tres sistemas celulares de la médula ósea esto es casos de panmielopatía proliferativa o panmielosis proliferativa policitemia + leucemia + megacariocitemia

En las microfotografías se podrá observar

1) elementos no maduros de la serie granulopoyética
2) elementos no maduros de la serie eritropoyética
3) megacariocitos en actividad trombocitopoyética

En las microfotografías sucesivas está documentada la proliferación mixta en el hígado y el bazo por medio de material obtenido por biopsia

En la sangre periférica se comprueba una pancitemia de 6 a 8 millones de glóbulos rojos por mm³ de 20 000 a 50 000 glóbulos blancos de 500 000 a 600 000 plaquetas

A veces durante el curso de la enfermedad uno de los tres sistemas puede adquirir una preponderancia absoluta que puede resultar pasajera o permanente en este segundo caso se habla de transmutación de una panmielosis en mielosis parcial que puede ser eritrémica (policitemia) o leucémica o megacariocítica

Es necesario subrayar la importancia de esta variabilidad de las manifestaciones clínicas de un mismo proceso morboso que se caracteriza siempre por la actividad proliferante de las células del tejido mieloide Se trata de distintas manifestaciones de una misma enfermedad

Sin embargo esta variabilidad de las manifestaciones clínicas durante el curso de la enfermedad *no es un fenómeno constante al contrario la marcha de las* enfermedades mieloproliferativas tiene casi siempre carácter de estabilidad *Por* ejemplo la leucemia mielógena crónica se desarrolla generalmente como forma pura con proliferación de los elementos de la serie granuloblástica sin participación de la serie eritrocitaria ni de la serie megacariocitaria La presencia en la sangre *periférica* de algún *eritroblasto ortocromático o* de algún *fragmento* de núcleo megacariocítico no es suficiente para sentar el diagnóstico de forma mixta Sólo en caso de que durante el curso de la leucemia mielógena crónica se observara el paso persistente en la sangre periférica de células de la serie eritrocitaria (proeritroblastos) y de la serie megacariocitaria (megacarioblastos) *profundamente* inmaduras se podría hablar de forma mixta (eritroleucemia leucomegacariocitemia) La biopsia de la médula ósea bazo e hígado debe confirmar el diagnóstico

Lo mismo puede decirse por cuanto respecta a la policitemia vera que no siempre es una panmielopatía Se pueden distinguir dos variedades de esta forma

a) Policitemia rubra vera que puede presentar durante mucho tiempo el cuadro hematológico clásico caracterizado por el aumento de los eritrocitos con *número normal de leucocitos* y plaquetas en *esta forma el proceso* proliferativo sólo afecta la serie eritrocitaria y queda circunscrito a la médula ósea sin interesar los órganos extramedulares

b) Policitemia vera mixta caracterizada por un cuadro hemático que es al mismo tiempo tanto cuantitativamente como cualitativamente eritrémico (aumento de eritrocitos y *presencia de proeritroblastos*) *leucémico (aumento*

tan una condicion inherente a las células mismas las cuales han adquirido una incapacidad para responder a las fuerzas que normalmente arreglan su prolifera cion y maduración Es ésta, de acuerdo con Furth, la alteración esencial de la leucemia y por lo tanto en estos casos de eritroleucemia es la alteracion esencial de las formas asociadas la eritremia y la *leucemia mielogena*

b) Presencia del proceso proliferativo eritroleucémico en todos los organos y tejidos y por ende no tan solo en la médula osea el bazo el hígado los ganglios linfaticos sino también en los riñones la glandula suprarrenal el testículo los pulmones el corazon etc

c) Irreversibilidad del proceso eritroleucémico

Estos caracteres anatomicos son muy evidentes en las microfotografías que reproducen los hallazgos de biopsias y examenes histológicos de un caso de eritroleucemia aguda

II *Caracteres hematologicos* —Al examen de la sangre periférica se observan las siguientes alteraciones morfologicas

a) *Simultanea presencia de elementos profundamente inmaduros y atípicos* de la serie eritropoyética (proeritroblastos, paraeritroblastos) y de la serie granulopoyética (mieloblastos paramieloblastos)

b) Variabilidad cuantitativa de estos elementos relativamente a las distintas fases de la enfermedad y las particularidades de cada caso

También estos caracteres hematologicos resultan muy evidentes en las micro fotografias que representan el cuadro hemático periférico del mismo caso de eritroleucemia aguda

III *Caracteres clínicos* —Las manifestaciones clínicas de la eritroleucemia aguda son las mismas manifestaciones típicas de la eritremia aguda y la leucemia mielogena aguda anemia grave de tipo progresivo hemorragias esplenomegalia *hepatomegalia fiebre irregular lesiones ulceronecroticas* de la cavidad bucal etc

Algunas veces hemos oído formular la duda de que la eritroleucemia no fuera sino una forma de leucemia con temporaneo aumento de eritroblastos en la médula osea Es facil demostrar la falta de fundamento de esta duda

1) la proliferacion eritroblastica no es un fenomeno temporáneo *sino persis* tente su duracion junto con la proliferacion granuloblástica sigue la duracion de la enfermedad eritroleucémica

2) la proliferacion eritroblastica no está circunscrita a la médula osea unica mente sino que se encuentra difusa en todos los organos junto con la prolifera cion granuloblástica

3) las alteraciones cualitativas de los eritroblastos en la eritroleucemia tienen los mismos caracteres que las alteraciones cualitativas de los granuloblastos como resulta tambien de los cultivos *in vitro* de la médula osea que han demos trado identicas anormalidades de maduracion asi en los eritroblastos como en los granuloblastos

No se trata pues de una leucemia con temporaneo aumento de eritroblastos ni tampoco de una eritremia con temporaneo aumento de granuloblastos sino de una proliferacion primitiva al mismo tiempo eritroblástica y granuloblastica generalizada

complejidad de la patogénesis de las formas mixtas y las formas evolutivas de las enfermedades mieloproliferativas en el hombre y los animales

Las conclusiones que podemos sacar después de la gran experiencia realizada en todos los países del mundo son las siguientes

1) Hay que reconocer la autonomía de entidades morbosas primitivas y bien definidas no solamente a las enfermedades mieloproliferativas puras tales como la leucemia mielógena y la policitemia vera sino también a las formas mixtas ya sea las de componente doble (eritroleucemia) o bien de componente triple (eritroleucomegacariocitemia)

2) En la evolución de las enfermedades mieloproliferativas se pueden tener pasos de una a otra forma, éstos deben considerarse como fases evolutivas de un unico proceso morboso que esencialmente es siempre el mismo pero que puede afectar en fases sucesivas los distintos sistemas celulares del tejido mieloide

3) Desde el punto de vista patogénico se puede pues hablar de unidad del proceso morboso con multiplicidad de manifestaciones anatomoclínicas dependientes ora de una distinta intensidad del estimulo causativo ora de una distinta capacidad de reacción del tejido afectado

Estas diferentes manifestaciones anatomoclínicas no son síndromes sino verdaderas enfermedades mieloproliferativas

Resumiendo las numerosas y bien documentadas observaciones especialmente las de estos últimos anos confirman el concepto de que los tres sistemas celulares del tejido mieloide (el eritroblástico el granuloblastico y el megacarioblastico) pueden proliferar no solo singularmente sino también *en masse o como unidad* como resulta del siguiente esquema

Unidad anatomica	tejido mieloide
Multiplicidad funcional	eritropoyesis granulopoyesis megacariopoyesis
Unidad patogénica	proliferación del tejido mieloide
Multiplicidad nosografica *Enfermedades mieloproliferativas*	*formas puras* formas mixtas formas de transición

Estos conceptos que yo expresé en un trabajo publicado en 1917 han sido ampliamente confirmados durante 35 anos de experiencia mundial

Erythroleukemia

Erythroleukemia is a primitive proliferative disease accruing from the myeloid tissue

The following table groups the different anatomo clinical forms of the proliferative diseases of the myeloid tissue

Partial Forms
(1) Leukemic myelosis
(2) Erythremic myelosis
(3) Megakaryocytic myelosis

Mixed Forms
(1) Erythroleukemic myelosis
(2) Leucomegakariocytic myelosis
(3) Erythromegakariocytic myelosis

de leucocitos y presencia de mieloblastos) y megacariocitémico (aumento de plaquetas y presencia de megacariocitos y megacarioblastos) En esta forma la autopsia pone de manifiesto una intensa proliferacion de los tres sistemas hema topoyéticos medulares con eritro gránulo y megacariopoyesis en el bazo hígado ganglios linfaticos riñones, pulmones páncreas cápsula suprarrenal meninges etc Jamás revela la autopsia esta proliferación extramedular en la policitemia rubra vera pura

En la mayoría de los casos la manifestacion de la leucemia fué consecutiva al tratamiento de la policitemia vera por los rayos rontgen o los isotopos radiactivos y por lo mismo fué atribuída una accion determinante a estos métodos terapéu ticos Indudablemente tienen gran importancia sin embargo no son factores indispensables ya que la panmielopatía proliferativa puede presentarse espon taneamente como ocurrió en dos casos por mí observados y en los que no se habia *practicado ni rontgenterapia ni isótopoterapia*

A veces la sucesión morbosa puede presentarse a la inversa durante un primer período que puede durar hasta más de un ano la enfermedad no es sino una leucemia granulocítica pura sigue un segundo período durante el cual a la leu cemia se agrega una policitemia vera dando así lugar a una enfermedad mielo proliferativa mixta *(leucemia con policitemia consecutiva)*

En su evolución la enfermedad puede llegar a una fase terminal en la que aumenta la proliferacion de los eritroblastos en la médula ósea pero disminuye su capacidad de dar origen a los globulos rojos maduros a consecuencia de esta disminuída produccion de eritrocitos maduros la poliglobulia queda substituída por la anemia con paso de eritroblastos en la sangre periférica Se establece asi otra variedad de enfermedad mieloproliferativa la eritroleucemia con anemia de evolucion mucho más rapida y menos benigna que la fase precedente de leu cemia con policitemia

Las causas de las distintas formas de las enfermedades mieloproliferativas son todavía obscuras Pueden depender ya de diferencias del factor causativo ya de diferencias de la reaccion del organismo como resulta también de las investi gaciones experimentales llevadas a cabo con el virus de las enfermedades mielo proliferativas de los pollos

Es sabido que estas enfermedades estan casi siempre representadas por una forma eritrémica pura muy raramente se observa la forma leucémica pura en muchas decenas de casos de eritremia espontanea de los pollos yo *he podido* observar tan solo uno de eritroleucemia Por la inoculacion del virus eritrémico en el embrion de pollo siempre se obtiene una forma eritrémica pura cuando el virus sea fresco pero cuando este mismo virus sufra cambios debidos a distintas condiciones (pasos en series conservación a ciertas temperaturas filtración etc) su inoculación puede provocar la *presentacion de una forma leucémica pura* Sin embargo ello no ocurre en todos los sujetos sino en un numero limitado provo cando en los demas como de costumbre la pre entacion de la forma eritrémica pura

Estos interesantes datos experimentales pueden servir para hacer entender la

studies by several groups is 5 formyl 5 6 7 8 tetrahydropteroylglutamic acid Leucovorin is acidic and forms di and tri equivalent alkaline earth salts It is stable at neutral or mildly alkaline pH for long periods of time even at temperatures of 55 C

The role of leucovorin in the clinic has developed as a rather orderly result of a wealth of investigations into the biological properties of this compound I shall attempt briefly to review some of these milestones before passing on to the clinical significance of leucovorin The hypothesis that leucovorin is one of the forms by means of which folic acid is metabolically active is substantiated by the fact that all species of bacteria and animals which require folic acid can thrive when leucovorin is substituted for folic acid A summary of the microbiological activity of leucovorin and related compounds for several lactic acid bacteria shows that leucovorin is active as a growth factor both for Le citrovorum and the folic acid requiring organisms S fecalis and L casei but that Le citrovorum differs from the latter organisms in that it responds only to large amounts of folic acid Similarly it has been shown that leucovorin may be used as a substitute to stimulate growth and hemopoiesis in both chicks and turkeys in these studies it was shown that leucovorin was considerably more active when injected than when administered in the diet

More dramatic still were the studies involving the ability of leucovorin to reverse the activity of the so called folic acid antagonists Leucovorin is about half as active as folic acid for growth of S fecalis and L casei however if aminopterin or one of the other folic acid antagonists is present in the culture medium leucovorin is now greatly superior to folic acid in reversing the growth inhibition Such data imply that leucovorin may be more closely related to the biologically active form of the folic acid enzyme than folic acid itself This finding that leucovorin can reverse the toxicity of aminopterin for microorganisms is paralleled by numerous studies showing this effect in fruit flies mice rats the chick embryo and monkeys That this may be a broad type of antagonism has been suggested recently in describing the antimalarial activity of some of the triazines

The relationship of leucovorin to the folic acid antagonists has been summarized by Karnofsky with the statement that the antagonists appear to be antileucovorin rather than antifolic This mechanism presumably involves leucovorin in two ways it interferes with the conversion of folic acid to leucovorin and it also competes with leucovorin in certain metabolic processes

Leucovorin has special significance for the clinician because it appears to be intimately related to the co enzyme form by means of which folic acid accomplishes its function in metabolic inter relationships It can be mentioned here only briefly that the folic acid leucovorin complex appears to be essential for the synthesis of nucleo proteins being needed particularly in the formation of thymidine This biological property is of utmost importance when rapid cell growth is involved as in erythropoiesis but also has a generalized importance for all cells

It would appear from all available evidence so far published that before PGA can carry out its catalytic functions in the cell it must first be converted to CF and that CF either represents the catalytically active form of PGA or is more closely related to this form than PGA itself This is supported by the finding of Nichol and Welch that there is an enzyme(s) in liver which can carry out this conversion There is considerable evidence now at hand suggesting that PGA (and presumably CF) catalyzes the formation of several amino acids including serine methionine and histidine and the purine and thymine moieties of the nucleic acids Isotope studies from many laboratories have established that formate or its biological equivalent is incorporated into these metabolites Thus the knowledge that CF contains a formyl group is of considerable biochemical interest for it provides a structural basis for the concept that CF is concerned with the transfer of the one carbon unit in metabolic reactions (If CF functions by shuttling a formyl group from one substrate to another it might be expected that tetrahydro PGA might have activity comparable to CF)

It is too early to predict the precise usefulness of leucovorin in clinical therapeutics but I hope to present a review of published reports as well as unpublished communications

Total Forms

(1) Erythroleukomegakaryocytic myelosis or panmyelosis

Study of chronic erythroleukomegakaryocytic myelosis (chronic panmyelosis) is very interesting because of the relationship of this disease with polycythemia vera. The former is a well defined disease with the following characteristics

Anatomical proliferation of the three bone marrow systems with extra medullary erythro leuco megakaryocyticpoiesis occurring in the spleen and liver lymphatic nodes kidneys lungs pancreas adrenals meninges etc

Clinical very marked splenomegaly moderate hepatomegaly and chronic evolution

Hematological erythrocytosis leucocytosis thrombocytosis with the presence of a small number of myeloblasts erythroblasts and megakaryocytes

In the evolution of chronic erythroleucomegakaryocytic myelosis any of the three varieties of cells may become predominant to the point of excluding the other two series and in these cases we speak of a turn towards a leukemic myelosis erythremic myelosis or megakaryocytic myelosis When there is an erythromegakaryocytic predominance the differential diagnosis from polycythemia vera is more difficult and a splenic biopsy or a spleen and liver puncture must be performed This shows in both organs the presence of normoblasts in different stages of evolution together with myeloblasts and megakaryocytes

Acute erythroleukemic myelosis is a well defined primary disease with the following characteristics

Anatomical proliferation of the medullary erythropoietic and leucopoietic systems with extra medullary erythro leucopoiesis (spleen lymphatic nodes liver kidneys adrenals stomach intestines thyroid hypophysis testicles myocardium etc) with a maturation arrest in the first phases of cellular evolution

Clinical severe anemia fever hemorrhagic manifestations hepatomegaly hypertrophy of the lymphatic nodes and chronic evolution

Hematologic erythrocytopenia erythroblastemia myeloblastemia and thrombocytopenia with erythremic and leukemic hiatus During the evolution of acute erythroleukemia an erythrocytic predominance (acute erythremia) or a leucocytic predominance (acute leukemia) can be observed sometimes

III communication 1

The Clinical Usefulness of Leucovorin

J M RUEGSEGGER*

During the past 4 years the establishment of a new member of the folic acid group has progressed rapidly through the process of recognition concentration isolation identification and synthesis The description by Sauberlich and Baumann of an unidentified growth factor required by Le citrovorum termed the citrovorum factor (CF) and the subsequent findings by a number of investigations that the CF was closely related chemically and biologically to folic acid (PGA) have called attention to the existence in natural materials of other forms of folic acid of biological and chemical interest Chemical investigations have shown that a synthetic compound variously termed leucovorin or folinic acid SF prepared by the reduction and formylation of PGA possesses the biological properties ascribed to the citrovorum factor The structure of this compound resulting from chemical

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studies by several groups is a formyl-5 6 7 8 tetrahydropteroylglutamic acid Leucovorin is acidic and forms di and tri equivalent alkaline earth salts It is stable at neutral or mildly alkaline pH for long periods of time even at temperatures of 55 C

The role of leucovorin in the clinic has developed as a rather orderly result of a wealth of investigations into the biological properties of this compound I shall attempt briefly to review *some of these milestones before passing on to the clinical significance of leucovorin* The hypothesis that leucovorin is one of the forms by means of which folic acid is metabolically active is substantiated by the fact that all species of bacteria and animals which require folic acid can thrive when leucovorin is substituted for folic acid A summary of the microbiological activity of leucovorin and related compounds for several lactic acid bacteria *shows that leucovorin is active as a growth factor* both for *L citrovorum* and the folic acid requiring organisms S fecalis and L casei but that L citrovorum differs from the latter organisms in that it responds only to large amounts of folic acid Similarly it has been shown that leucovorin may be used as a substitute to stimulate growth and hemopoiesis in both chicks and turkeys in these studies it was shown that leucovorin was considerably more active when injected than when administered in the diet

More dramatic still were *the studies involving the ability of leucovorin to reverse the activity of the so called folic acid antagonists Leucovorin is about half as active as* folic acid for growth of S fecalis and L casei however if aminopterin or one of the other folic acid antagonists is present in the culture medium leucovorin is now greatly superior to folic acid in reversing the growth inhibition Such data imply that leucovorin may be more closely related to the biologically active form of the folic acid enzyme than folic acid itself This finding that leucovorin can reverse the toxicity of aminopterin for microorganisms is paralleled by *numerous studies showing this effect in fruit flies mice rats* the chick embryo and monkey That this may be a broad type of antagonism has been suggested recently in describing the antimalarial activity of some of the triazines

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which may point the way to a clearer delineation of the role of this compound in human physiology Numerous investigators have used leucovorin without detecting any evidence of toxicity to the human organism all routes of administration have been used with preference being shown for intramuscular injection until the role of leucovorin is known more precisely it is suggested that this compound continue to be administered by the same route

The megaloblastic anemia of infancy has been reported by Woodruff et al to be remitted by large doses of leucovorin given parenterally moreover a secondary reticulocytosis did not follow the subsequent administration of B_{12} and folic acid

Spies and his associates were probably the first to demonstrate the usefulness of leucovorin in the treatment of sprue They administered 3 mg daily for 10 days and obtained excellent results with maximal clinical and hematopoietic results

These same workers treated megaloblastic anemia associated with nutritional deficiency with satisfactory results after administering fairly large doses of leucovorin Watson also demonstrated the efficacy of this compound in treating a patient with nutritional anemia who had not responded to the administration of large doses of B_{12} intramuscularly

Limited experience with a group of other macrocytic anemias has not disclosed positive results with leucovorin For instance case reports have been received in which leucovorin failed to improve the anemia associated with carcinoma with cirrhosis or cases of aplastic anemia On the other hand Rottino noted an increase in the hemoglobin and erythrocytes of patients with the anemia frequently associated with Hodgkin s disease in the e cases improvement did not follow leucovorin therapy except after the addition of aureomycin and adenylic acid Similarly Hunter has reported equivocal but encouraging results after the administration of leucovorin to a group of patients with anemia refractory to conventional anti anemia therapy

While folic acid is not recommended for the treatment of Addisonian pernicious anemia several reports have appeared in the literature which demonstrate the hemopoietic activity of leucovorin in pernicious anemia Quantitative comparisons of leucovorin and folic acid in pernicious anemia indicate that leucovorin is no more active than folic acid in producing a reticulocyte re ponse Davidson and Girdwood observed submaximal reticulocytosis after single doses of 3–12 mg intramuscularly but remarked on the improvement in well being of each of their patients in spite of the small do es

Probably more striking has been the occasional report of patients who had neurologic complications of pernicious anemia Meyer and associates told of the administration of 3–6 mg daily for two months to a patient with pernicious anemia of 14 years duration leg burning ceased while leucovorin was being given Similarly in another patient the Babinski phenomenon disappeared after 6 weeks of daily dosage of 3 mg of leucovorin In another report Meyer and Diefenbach described the treatment of a pernicious anemia patient who had paresthesiae of the hands and feet and diminished vibratory sensation of both ankles Six mg of leucovorin daily by mouth produced a submaximal reticulocytosis and great subjective improvement within 7–10 days The paresthesiae disappeared but reappeared at the end of 3 months when the daily intake was reduced to 3 mg

Moore and colleagues report a similar case with even more dramatic improvement in a pernicious anemia patient with severe hematologic and neurologic involvement Good hematologic response followed the administration of 10 mg of leucovorin daily by mouth neurologic remission was so complete by the end of 6 weeks that the daily dosage was reduced to 5 mg Neurologic regression became apparent on that dosage but improvement recurred when the daily dosage was restored to 10 mg Final conclusions concerning the usefulness of leucovorin in pernicious anemia must await considerably more numerous trials in this disease

The limited usefulne s of the folic acid antagonists in the treatment of acute leukemia and certain neoplastic diseases has been well established in many clinics in the western hemisphere as well as in certain research centers of Europe While this group of compounds is capable of effecting a temporary remission in many cases of acute leukemia especially of childhood their usefulness in many instances has been nullified by the early appearance

of signs of toxicity such as mucosal ulceration hemorrhage and alopecia The margin of safety with these compounds varies considerably from patient to patient this difference may depend on the folic acid nutrition of the patient a factor not easy to evaluate clinically Burchenal and his colleagues showed that the toxicity of aminopterin for mice can be reversed by leucovorin but that a time factor as well as a dosage factor exists Schoenbach Greenspan and Colsky first confirmed this reversibility in the clinic when they demonstrated the efficacy of leucovorin in overcoming the toxicity of aminopterin and one of its analogs Whether this may become a practical application to successful folic acid antagonist therapy will depend on future carefully controlled observations It undoubtedly will be a delicate task to steer a safe course between complete antagonism on the one hand and the danger of toxicity on the opposite side However sufficiently numerous reports have appeared in the literature to warrant the simultaneous or almost simultaneous administration of leucovorin and the folic acid antagonists In addition to the published reports Cartright Bethell Hunter Vilter and Wilson have described their experiences as encouraging in employing leucovorin in preventing or counteracting the toxicity which frequently follows folic acid antagonist therapy

Moreover on a theoretical basis at least leucovorin should be employed as an antidote following the inadvertent overdosage with the folic acid antagonists In such an instance the time factor would be crucial and leucovorin should be administered in large doses as quickly as possible after the detection of the mistake

The role of leucovorin in stimulating healing of wounds has not been explored One group of investigators has reported personally that the weeping lesions of psoriasis and mycosis fungoides appear in a limited number of cases to dry and to undergo regeneration rapidly following the administration of leucovorin intramuscularly Such observations require confirmation but deserve repetition in view of the various theses concerning the function of folic acid metabolism in the human organism

La Significación Clínica de la Leucovorina

Leucovorina es el nombre que se le da a un factor necesario para el crecimiento del Leuconostoc citrovorum los sinónimos incluyen factor citrovorum FC y ácido folínico Está muy estrechamente vinculado al ácido fólico y puede que inclusive sea la forma en la cual el ácido fólico es metabólicamente activo en muchos animales Aunque no se conoce todavía con toda exactitud su fórmula estructural algunos opinan que la Leucovorina es el ácido 5 formil 5 6 7 8 tetrahidropteroil glutámico

Siendo la forma metabólicamente activa del ácido fólico la Leucovorina se ha demostrado ejerce los mismos efectos en el organismo humano que los que se notan durante la administracion del ácido folico De aquí el que se haya visto producir una remisión clínica y hematológicamente satisfactoria en los tipos principales de anemia megaloblástica Casos clínicos serán discutidos por medio de proyecciones para demostrar el efecto de dicha substancia en casos selectos

Probablemente sus resultados clínicos más dramáticos pueden ser vistos en el uso combinado de Leucovorina con los antagonistas del ácido folico La Leucovorina es un antagonista muy potente de la Aminopterina y otros miembros del grupo de compuestos antagonistas del ácido fólico Como resultado de la confirmacion de la eficacia de este grupo de compuestos en la prolongacion de la vida en la leucemia y en unas pocas enfermedades vinculadas con la leucemia la Leucovorina ha adquirido importancia clínica para aliviar la toxicidad de estos compuestos químicos asimismo la Leucovorina queda como un antídoto efectivo en casos de dosis excesivas por inadvertencia con los antagonistas del ácido fólico

Otros usos de la Leucovorina serán discutidos en conexion con su papel en la nutrición celular

III communication 2

Effect of 4 Amino Pteryol Glutamic Acid (Aminopterin) on White Rats

J FERRER J T LEWIS S L RABASA and A D DOMENICO*

The object of this work is to contribute to the knowledge of the pharmacologic effects and mechanism of action of aminopterin

Acute and chronic intoxications were produced In the acute ones a single dose varying between 100 and 800 µg per 100 Gm of body weight was given The LD50 was 163 ± 1 8 µg per 100 Gm for females and 315 7 ± 1 32 µg per 100 Gm for males This difference being highly significant Another series of animals received a dose of 5 µg per 100 Gm daily until death a dose equivalent to that used in human therapeutics The lethal dose was also significantly smaller in females than in males

During the first days following the administration of the drug no abnormal signs appear In the chronic intoxication the first sign is a change in the blood picture while in the acute type it is a loss of weight

Aminopterin exerts its effects on different groups of the bone marrow cells The first to be affected are the erythroblasts Due however to the long duration of the erythrocytes in the circulation anemia does not become apparent until a later stage Aminopterin acts upon the neutrophile and eosinophile families in two stages In the first it has a stimulative effect in the second it depresses the production of these cells until they disappear completely from both the blood and the bone marrow The eosinophils show this process later and some animals die without the disappearance of this family In animals which survive after a severe acute intoxication reappearance of neutrophils is the first sign of recovery Later they rise above the initial level Lymphocytes begin to decrease in the blood before signs of severe intoxication appear

The water intake and urine excretion increase to several times their previous level *No glycosuria appears and the NaCl intake was not increased*

This syndrome appears towards the end of chronic intoxication Animals with acute intoxication show it when the intoxication is neither too severe nor too mild Oral lesions appear late in both types of intoxication Diarrhea was significantly correlated to severity of intoxication in the acute cases but was seldom seen in animals with chronic intoxication

Towards the end of chronic intoxication a highly correlated series of symptoms is observed disappearance of neutrophils diabetes insipidus loss of weight and anorexia Lymphopenia is weakly correlated with this syndrome Anemia and the increase of neutrophils are not correlated at all with this syndrome

Necropsy The adrenals were hypertrophied This fact is due to an enlargement of the cortex and to the intense congestion and hemorrhagia of the whole gland

The thymus was atrophied Microscopic examination showed it to be reduced to a few lymphoid cells in the midst of a sclero adipose tissue Atrophy of the Malpighian corpuscles *and of the lymph nodes were also seen Frequently* infarcts were seen in the spleen which on microscopic examination were shown to be due to a perivascular lesion Around the capillaries a granular basophilic substance without structure is deposited The lesions were also seen sometimes in the lymph nodes and the myocardium The liver was enlarged and showed a fatty degeneration

EFECTOS DE LA AMINOPTERINA SOBRE LA RATA BLANCA

La DL 50 para una sola inyeccion fué de 163 ± 1 8 microgramos por 100 gr en hembras y 315 7 ± 1 3? en machos La inyeccion de 5 microgramos por 100 gr diariamente también demostro la menor resistencia en las hembras En el cuadro sanguineo se vio primero excita

* Instituto de Investigaciones Médicas Rosario Argentina

ción y luego depresion de los neutrofilos desapareciendo éstos en la intoxicación grave En los eosinófilos las fases de excitación y depresión se observaron más tardiamente La linfopenia se vió antes de que aparecieran los signos de la intoxicacion grave Los exámenes de la médula ósea hechos en serie demostraron que los elementos de la serie roja son los primeros en ser afectados pero la anemia sólo se vió después de haberse prolongado algun tiempo el tratamiento y fué marcada en los animales que resistieron mucho En el período final se vió un sindrome caracterizado por anorexia pérdida de peso diarrea (sobre todo en la intoxicacion aguda) desaparicion de los neutrofilos disminución de los eosinofilos poliuria sin glucosuria Se observo estomatitis (gingivitis y caries de los incisivos) en una fase tardía En la autopsia se vió hipertrofia de las suprarrenales por aumento de la capa fasciculada de la corteza atrofia marcada del timo de los corpusculos de Malpighi del bazo y de los ganglios linfáticos Se vieron infartos en el bazo en los ganglios linfáticos y en un caso en el miocardio debidos a una lesión perivascular que impermeabiliza el capilar sin obstruir su luz Había marcada degeneración granulogra osa del hígado

III-communication 3

The Role of the Adrenals in the Effect of 4 Amino Pteroyl Glutamic Acid (Aminopterin)

J T LEWIS S J RABASA J FERRER and A D DOMENICO*

The adrenals were removed from adult white rats and 48 hours later aminopterin in doses of 5 micrograms per 100 g of body weight per day was injected intraperitoneally until the animals died The lethal dose was significantly lower than for animals with intact adrenals There was no sexual difference in resistance to the drug since the lethal dose for males was 66 ± 8 5 microgram per 100 Gm and 64 ± 5 5 μg per 100 Gm for females

The blood picture and the bone marrow showed the same disturbances as were seen in animals with intact adrenals treated with aminopterin None of the adrenalectomized animals had diabetes insipidus like syndrome typical of the final stages of aminopterin intoxication in normal rats

The autopsy revealed that the weight of the thymus was slightly below that of normal non treated animals but the difference was not significant There was no marked atrophy as was provoked by aminopterin in animals with intact adrenals and there was no hypertrophy of the thymus as in the adrenalectomized nontreated controls Microscopic examination showed a normal aspect of the thymus There was no fatty degeneration of the liver

The adrenals therefore play an important part in the resistance to this as to many other toxic substances they are responsible for the greater part of thymolitic effect of aminopterin and they are necessary for the production of the diabetes insipidus like syndrome and fatty degeneration of the liver On the other hand aminopterin has a certain thymolitic action and exerts all its effects on the bone marrow without the mediation of the adrenals

Another series of rats of both sexes half of which were adrenalectomized were treated with aminopterin as in the preceding experiment They were divided into four groups which were given (a) aminopterin alone (b) aminopterin plus cortisone (c) aminopterin plus desoxicorticosterone acetate (DOCA) and (d) aminopterin plus both adrenal steroids The results showed once again the sexual difference in resistance and the hypersensitiveness caused by adrenalectomy Treatment with the adrenal steroids had no significant effect on the resistance to aminopterin but DOCA had a small protective effect

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III communication 2

Effect of 4 Amino Pteryol Glutamic Acid (Aminopterin) on White Rats

J FERRER J T LEWIS S I RABASA and A D DOMINICO*

The object of this work is to contribute to the knowledge of the pharmacologic effects and mechanism of action of aminopterin

Acute and chronic intoxications were produced In the acute ones a single dose varying between 100 and 800 μg per 100 Gm of body weight was given The LD50 was 163 ± 1 8 μg per 100 Cm for females and 315 7 ± 1 32 μg per 100 Gm for males This difference being highly significant Another series of animals received a dose of 5 μg per 100 Gm daily until death a dose equivalent to that used in human therapeutics The lethal dose was also significantly smaller in females than in males

During the first days following the administration of the drug no abnormal signs appear In the chronic intoxication the first sign is a change in the blood picture while in the acute type it is a loss of weight

Aminopterin exerts its effects on different groups of the bone marrow cells The first to be affected are the erythroblasts Due however to the long duration of the erythrocytes in the circulation anemia does not become apparent until a later stage Aminopterin acts upon the neutrophile and eosinophile families in two stages In the first it has a stimulative effect in the second it depresses the production of these cells until they disappear completely from both the blood and the bone marrow The eosinophils show this process later and some animals die without the disappearance of this family In animals which survive after a severe acute intoxication reappearance of neutrophils is the first sign of recovery Later they rise above the initial level Lymphocytes begin to decrease in the blood before signs of severe intoxication appear

The water intake and urine excretion increase to several times their previous level No glycosuria appears and the NaCl intake was not increased

This syndrome appears towards the end of chronic intoxication Animals with acute intoxication show it when the intoxication is neither too severe nor too mild Oral lesions appear late in both types of intoxication Diarrhea was significantly correlated to severity of intoxication in the acute cases but was seldom seen in animals with chronic intoxication

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III communication 4

The Endogenous Factor in the Leukemias (The Leukemias as Diseases of Adaptation)

FELIPE JIMENEZ DE ASUA*

The external agents would be the causes unleashing reversible abnormal reactions (leukemoid reactions) or irreversible reactions (leukemias) in organisms with a lympho myeloid imbalance produced by endocrine dysfunction and especially in all concerned acute leukemias by disturbances of the pituitary corticoadrenal system (constitutional hypoadrenal conditions) The familial leukemias would not be the expression of the heredity of the disease as such but would be the expression of the heredity of the soil

The corticoadrenal secretions and indirectly the adrenocorticotrophic hormone would curb the activity of the most undifferentiated cells of the hematopoietic apparatus and stimulate the orthoplastic maturation especially of the myeloid system

In this sense such regulating hormones would be identifiable with Miller s myelokentric and lymphokentric acid and with the hypothetical natural antileukemic antibodies of the French authors

Arguments are presented favoring these hypotheses that would allow the inclusion of the leukemias in the group of diseases of adaptation

Finally the results obtained with the combined treatments in 50 cases of acute leukemias are related In children the remissions reach 66 per cent and the average survival of the cases that respond favorably is over a year

El Factor Endógeno en las Leucemias (Las Leucemias como Enfermedades de Adaptación)

Los agentes externos serían causas desencadenantes de reacciones anormales reversibles (reacciones leucemoides) o irreversibles (leucemias) en organismos con un desequilibrio linfo mieloide ocasionado por disfunción endocrina y especialmente por lo que a las leucemias agudas se refiere por trastornos del sistema pituitario córtico adrenal (estados de hipo adrenia constitucional) Las leucemias familiares no serían expresión de herencia de la enfermedad como tal sino de herencia del terreno

Las secreciones córticoadrenales e indirectamente la hormona adrenocorticotropa frenarían la actividad de las células más indiferenciadas del aparato hemopoyético y estimularían la maduración ortoplástica especialmente del sistema mieloide

En este sentido tales hormonas reguladoras serían identificables con los ácidos mielokéntrico y linfokéntrico de Miller y con los hipotéticos anticuerpos naturales antileucémicos de los autores franceses

Se presentan argumentos en favor de estas hipótesis que permitirían incluir las leucemias en el grupo de enfermedades de adaptación

Finalmente se refieren los resultados obtenidos con los tratamientos combinados en 50 casos de leucemia aguda En los niños las remisiones se elevan a 66% y la supervivencia media en los casos que responden favorablemente es superior al año

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*Servicio de Hematología del Centro Gallego Buenos Aires Argentina

A third series was therefore injected aminopterin in the usual way and treated with 200, 400 and 800 µg per 100 Gm daily of DOCA. The resistance of the adrenalectomized rats rose to the normal level, the protective effect increasing with the dose. In rats with intact adrenals DOCA *also had a protective effect, increasing resistance to aminopterin.*

Cortisone had the same effect on the thymus as the adrenal of the animal stimulated by aminopterin both in its degree and statistical significance. It also had an inhibitory effect on the weight of the spleen which was not exerted by the animals own adrenal. DOCA had no effect on the thymus; it neither potentiated nor inhibited the action of aminopterin. Cortisone had a small inhibitory effect on adrenal hypertrophy provoked by aminopterin; the decrease in adrenal weight was *proportionally the same as that provoked by* the same dose of cortisone in normal rats.

The diabetes insipidus-like syndrome was seen in 18 out of 24 rats with intact adrenals treated with aminopterin; it did not occur in any of the adrenalectomized treated with aminopterin but not with adrenal steroids; it was seen in 5 out of 18 adrenalectomized rats treated with aminopterin and one or other or both the adrenal steroids. Animals which were given larger doses of DOCA had polyuria and an increase in the intake of NaCl before the symptoms corresponding to the final stage appeared. Later NaCl intake diminished without a decrease in the intake of water or the urinary output. In some animals after a first period of polyuria with increased NaCl intake there was an interval of normal diuresis followed by a second period of polyuria without an increase of NaCl intake coinciding with symptoms of the final stage, e.g., agranulocytosis. The diabetes insipidus-like syndrome provoked by aminopterin requires therefore a certain level of adrenal function for its appearance. This level can be substituted in adrenalectomized animals by small doses of cortisone or DOCA. The syndrome, however, is not caused by hyposecretion of adrenal steroids, nor by the adrenal mechanism that regulates sodium metabolism.

The serum of rats in the final stages of aminopterin intoxication with a well developed diabetes insipidus has the same or even a higher antidiuretic activity as serum of normal animals. Aminopterin therefore does not provoke diabetes insipidus by inhibiting the *secretion of the antidiuretic hormone of the hypophysis. Probably it acts directly on* the renal tubes blocking the effect of this hormone, but a certain amount of the adrenal diuretic factor is necessary for the occurrence of polyuria.

Aminopterin was injected intraperitoneally and blood counts were made 3, 6, 9 and 24 hours after. There was an increase in neutrophils together with lymphopenia and eosinopenia within the first 9 hours with a return to normal at 24 hours. Adrenalectomized animals had the same degree of neutrophilia after the injection of aminopterin, but the decrease in lymphocytes and eosinophils was significantly smaller. Aminopterin therefore has an effect similar to that of ACTH but it would be premature to conclude that it provokes adrenal hypertrophy exclusively by stimulating the secretion of corticotrophin.

Papel de las Suprarrenales en los Efectos de la Aminopterina

La suprarrenalectomía bilateral aumentó la sensibilidad de las ratas blancas al efecto tóxico de la aminopterina ($p < 0.01$). En las suprarrenoprivas se observaron los mismos efectos sobre la sangre y la médula ósea que en las normales tratadas con aminopterina, pero no se observó la diabetes insípida del período terminal. El timo tenía un peso y aspecto histológico normales, no había la hipertrofia observada en los testigos suprarrenoprivos no tratados ni la atrofia vista en los normales tratados. No había degeneración grasa del hígado. La cortisona no protegió contra el efecto tóxico de la aminopterina en dosis (1.25 *mg/100 g/día*) que produjo atrofia del timo y del bazo e inhibió parcialmente la hipertrofia de las suprarrenales. El acetato de desoxicórticosterona (DOCA) aumentó la resistencia a la aminopterina de las normales y elevó la de las suprarrenoprivas al nivel de aquellas. Se observó la poliuria final en algunas suprarrenoprivas tratadas con cortisona o DOCA. Dentro de las 9 horas después de inyectar una dosis de aminopterina se observó neutrofilia, eosinopenia y linfopenia en las normales. En las suprarrenoprivas faltó la eosinopenia y la linfopenia fué menor.

III communication I

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III communication 5

Virulence Variations of Leukemic Lines Produced by Several Transfers in Foreign Hosts

G HOECKER O PIZARRO D BRNCIC and G GASIC*

For the better understanding of the relationship between hosts and transplantable leukemic lines an investigation was done on the behavior of some leukemic lines in foreign hosts after one two and up to three successive passes The results indicate that the virulence of these lines may diminish or increase after the first pass depending on the combination of hosts and leukemic lines and also in some combinations depending on the length of time that the leukemic line lived in the different strains of mice The authors also investigated the role played by antibodies and hypothetical aiding substances in these situations Although these substances may explain the increase in virulence observed in some cases they certainly do not explain the cases where there is a decrease or irregular changes of the virulence In these cases it is probable that the antibodies may act in a first phase by diminishing the virulence and increasing it afterwards either by a selective action which favors the growth of the pre existent and more resistent cellular types or by inducing genetic changes in the cytoplasm favoring the development of plasmogenes which were present only in a potential state

VARIACION DE LA VIRULENCIA DE LAS LEUCEMIAS PROVOCADAS POR PASAJES SUCESIVOS POR DISTINTOS HUESPEDES

Haciendo varias transferencias de una sola línea leucémica a través de *huéspedes de* distintos geno tipos y variada suceptibilidad se observaron variaciones de virulencia de esta línea aumentando en ciertos tipos de ratones y disminuyendo en otros El posible mecanismo de tales cambios será discutido

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This work has been carried out with partial aid granted by the Rockefeller Foundation for Medical Research

III communication 6

17 Ketosteroid Excretion in Leukemia

HERNAN ACEVEDO RAUL ECHEVERRY and CARLOS GUZMAN E †

The total urinary 17 ketosteroid (17 KS) output of leukemic subjects males and females has been studied Insofar as was possible urine specimens were taken before any kind of treatment was instituted

The determinations were made with a standard method used in our laboratory Without going into details it is to be noted that the fractionation procedure included treatment with Girard s Reagent T in order to separate ketocompounds and to eliminate non ketonic chromagens

The results obtained show that markedly subnormal quantities were excreted by these

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patients Till now in our cases we have not seen a correlation between the type of disease and the excretion levels

It is concluded that in this disease an alteration of the adrenal functionalism exists at least in the production of these catabolites This alteration leads us to think in the possibility of an hypofunction of at least a part of the adrenal cortex

EXCRETIÓN DE 17 CETOESTEROIDES EN LEUCEMIAS

Se estudió la excreción de 17 cetoesteroides neutros totales urinarios (17 CE) en un grupo de enfermos leucémicos de ambos sexos Hasta donde fué posible las muestras de orina fueron recolectadas antes de que cualquier tipo de tratamiento fuera instituído

Los análisis se efectuaron según el método standard empleado en nuestro laboratorio Sin entrar en detalles es conveniente hacer notar que el proceso de fraccionamiento incluye un tratamiento con reactivo de Girard T con el objeto de separar los cetocompuestos y eliminar las sustancias cromógenas

Los resultados obtenidos en ambos sexos indican una disminución clara en la excreción de estos catabolitos Hasta el momento en el número de casos estudiados no existe una correlación entre el tipo de leucemia y los niveles de excreción

Se concluye que en esta enfermedad existe una alteración del funcionalismo adrenal al menos en lo que a producción de estos catabolitos se refiere Tal alteración permite pensar en la posibilidad de una hipofunción de por lo menos una parte de la corteza suprarrenal

III communication 7

Our Experience in the Treatment of Leukemias

ANGELO BASERGA*

As treatment of leukemia can not be done according to its etiology a pathogenetic type of treatment is employed All the drugs used with success in the treatment of leukemias are inhibitors of hematopoiesis In animals aplastic pictures can be obtained by giving strong doses of these medications Alterations caused by reduction of cell division up to the appearance of giant cells of myeloid origin may also occur The sensitivity of leukemic cells to the inhibitors is nevertheless stronger than that of normal marrow cells it is then possible to do a differential cytochemotherapy considering the relationship of toxic doses for leukemic cells and for normal cells The sensitivity of the different leukemic cells also varies in different cases and in different phases in one same patient That is why it is necessary to elect individually the best drug In our experience radio therapy in its different forms of application is the treatment of choice in the chronic forms and the anti vitamins and ACTH in the acute forms these last cases responding differently according to the age and clinical and hematological picture The cases which are rapidly progressing respond better initially with ACTH and the ones that are less progressive benefit from the folic acid antagonists These last drugs can also be used in association with ACTH The hormonal treatment can sometimes delay the body s resistance to the anti folic drugs permitting to obtain longer remission (up to 6 months in our cases)

NUESTRA EXPERIENCIA EN EL TRATAMIENTO DE LAS LEUCEMIAS

No pudiendo efectuar un tratamiento etiológico en las leucemias se hace un tratamiento patogenético Todos los medicamentos usados con éxito en el tratamiento de las leucemias son inhibidores de la hematopoyesis en los animales puede obtenerse con fuertes dosis

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cuadros aplásticos medulares y alteraciones determinadas por detención de la división celular hasta la aparición de células gigantes de origen mieloide (células megamieloides). La sensibilidad de las células leucémicas a estos inhibidores es sin embargo mayor que la sensibilidad de las células medulares normales; es así posible hacer casi una *citoquimioterapia diferencial* y se podría así llegar a calcular un *index citoquimioterapico* en relación de la dosis tóxica para las células leucémicas y para las células normales. La sensibilidad de las diferentes células leucémicas es aun muy diferente en los distintos casos y en las *diferentes fases* de un mismo enfermo; por eso es necesario elegir individualmente el mejor medicamento.

En nuestra experiencia la *radioterapia* en sus diferentes modalidades queda como el tratamiento de elección en las formas crónicas, y las antivitaminas y el ACTH en las formas agudas; también en estos casos con diferencias según la edad, formas hematológicas y clínicas. Las formas con evolución más rápida pueden obtener alguna ventaja preferentemente con el ACTH; las de evolución menos violenta pueden constituir una indicación de los antagonistas del ácido fólico. Se pueden también usar asociados el ACTH y los antifólicos, pudiendo a veces el primero retardar la resistencia a los antifólicos y obteniendo también a veces en las leucemias agudas remisiones más o menos largas (hasta de seis meses en nuestras observaciones).

III communication 8

Effects of Triethylenmelamin (TEM)

LUDWIG HEILMEYER*

TEM has been used systematically in 50 cases of leukemia. In 80% of chronic myeloid and chronic lymphatic leukemias results have been favorable.

Remissions lasted from 4 weeks to 8 months. To obtain remissions the doses necessary varied surprisingly between a total from 15 to 370 mg, that is twentyfold. For each individual patient the effective dose has to be "titrated." Some rare cases showed an extreme sensitivity towards TEM. In these cases total doses of 15–20 mg in 3–6 days were sufficient to normalize the blood picture for weeks and months and to keep tumors of the spleen under control. In these highly sensitive cases TEM is by far the best therapeutic and much more effective than x rays. Relapses disappeared promptly after renewed TEM therapy. No dangerous side effects could be observed.

Transient leukopenias recovered promptly. In acute leukemias TEM is no better than the usual method of treatment. In the blood picture of healthy individuals 3 x 5 mg TEM produces no significant change except a slight transient depression of leukocytes. In rats big doses of TEM lead to leukopenia. The lymphocytes are here more affected than the granulocytes. A slight depression of hemoglobin and platelets is inconstant, although in one case a complete aplasia of the bone marrow was found. Rats treated for 6 weeks with TEM in a total dose of 66–110 mg/kg have been histologically examined (Pathol. Institut Freiburg, Doz. Dr. Altmann). In some cases a total reduction of lymphatic tissues was found. The structure of liver, kidney, pancreas and parotids was absolutely normal. Only the testicles showed an intensive disturbance of the spermiogenesis. Therefore TEM seems to be applicable even in high doses also in men.

Efectos de la Trietilenmelamina (TEM)

TEM ha sido usado sistemáticamente en 50 casos de leucemia. En un 80% de las leucemias mieloides crónicas y leucemias linfoides crónicas los resultados han sido favorables (pro-

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bado por varios preparados) Las remisiones duraron de 4 semanas a 8 meses Para obtener remisiones la dosis necesaria varió sorprendentemente de un total de 15 a 370 mg ésto es veinte veces más Para cada paciente la dosis efectiva debe ser determinada en pocos casos se mostró una sensibilidad extrema hacia el TEM En esos casos la dosis total de 15 a 20 mg dados en 3 a 6 días fueron suficientes para normalizar el cuadro sanguíneo por semanas y meses y para mantener los tumores del bazo bajo control En estos casos altamente sensibles el TEM es mucho más efectivo que los rayos X Las recaídas desaparecían rápidamente después de un nuevo tratamiento con TEM Efectos secundarios peligrosos no han sido observados Las leucopenias transitorias recobraron rápidamente En leucemias agudas el TEM no es más efectivo que los tratamientos usuales En cuadros de sangre normales 3–5 de TEM no producen cambios significativos aparte de una pequeña y transitoria depresión de los leucocitos En ratas una gran dosis de TEM lleva a la leucopenia En este caso son más afectados los linfocitos que los granulocitos Se observa una pequeña depresión de la hemoglobina y de las plaquetas Sin embargo en un caso se halló una completa aplasia de la médula

Ratas tratadas durante 6 semanas con TEM con una dosis total de 66–110 mg/kg fueron examinadas histológicamente (Pathol Inst Friburgo Doz Dr Altmann) En algunos casos se encontró una reducción total de los tejidos linfáticos La estructura del hígado riñones páncreas y parótida eran completamente normales Sólo los testículos mostraron un intenso trastorno de la espermiogénesis Por lo tanto puede aplicarse el TEM en grandes dosis incluso en los hombres

III communication 9

Nuestra Experiencia en el Tratamiento de los Leucémicos

LUIS DE MARVAL y ALBERTO S SANTORO*

No es nuestro propósito efectuar un estudio crítico de las medicaciones empleadas en el tratamiento de la leucemia Diremos en cambio cómo luego de muchos años de seguir estos enfermos encaramos la forma de conducirlos Estamos convencidos de que hoy por hoy siendo la leucemia incurable no debemos obstinarnos en estériles tentativas de erradicación Por eso no combatimos la enfermedad más allá del límite que permita al enfermo sobrellevarla lo mejor posible

Es para nosotros de la mayor importancia cuidar el estado general del enfermo Recordemos que el leucémico es frágil fácil presa de afecciones que se ceban en un organismo de defensas disminuídas Todo cuanto hagamos para mejorar esas defensas prolongará la vida del enfermo Por eso consideramos que el reposo la buena alimentación la protección contra las intercurrencias y el alejamiento de las angustias materiales y espirituales son del mayor valor en el sostenimiento de estos enfermos

Nosotros seguimos muy de cerca a nuestros leucémicos estudiándolos de continuo listos para actuar si fuera necesario Pero nunca apresuramos nuestra intervención porque hemos visto leucemias remitir espontáneamente Por otra parte todos los medicamentos citolíticos que podemos emplear tienen una acción depresora sobre el organismo y a veces importan una verdadera agresión contra el mismo

Empleamos los medios terapéuticos que más conocemos y que mejores resultados nos han dado La Roentgenterapia el arsénico y la hemoterapia constituyen para nosotros todavía el trípode de la terapéutica antileucémica La radioterapia que hemos empleado personalmente en el Instituto Municipal de Hematología de la Ciudad de Buenos Aires nos ha deparado muchas satisfacciones casi sin accidentes La consideramos el tratamiento

Buenos Aires Argentina

de elección para las mielosis crónicas y para las hiperplasias ganglionares crónicas de las linfosis Somos cautos en la dosificación pués hemos aprendido a esperar el resultado alejado de las irradiaciones Una sola irradiación sobre bazo puede provocar una remisión duradera ocasionalmente hasta más de un ano en mielosis crónicas vírgenes de tratamiento No tenemos pués esquemas terapéuticos usamos la radioterapia en la dosis mínima util

Al arsénico utilizado en dosis medias ininterrumpidas le confiamos el sostén de las remisiones leucémicas Tenemos la convicción de que aleja las recidivas

La hemoterapia en toda la gama de sus posibilidades actua como elemento sustitutivo y aporta elementos que aun no conocemos Suele provocar remisiones algunas veces espectaculares en las leucemias agudas y puede solucionar temporalmente las anemias y hemorragias de las crónicas

Todo ésto no significa que menospreciemos el valor de otras medicaciones en el tratamiento de esta enfermedad Las usamos como coadyuvantes o supletorias Entre ellas el uretano nos ha dado buenos resultados en las formas cronicas No tenemos suficiente experiencia en isotopos radioactivos para poder formarnos una opinión personal En las leucemias agudas hemos visto remisiones con antifólicos y hormonas pero los resultados no son constantes La posibilidad de lograr remisiones postransfusionales hace que transfundamos generosamente a estos enfermos

Estas observaciones derivadas de nuestra experiencia personal coinciden con las de muchos hematologos casi diríamos con la mayoría de los que han tratado muchos leucémicos durante largos años Pero como gran parte de estos enfermos son tratados por médicos generales importa qué desde esta plataforma que polariza la atencion médica internacional se reafirmen las directivas terapéuticas generales bien asentadas por la experiencia y tambien los consejos que repriman una excesiva agresividad de los tratamientos citolíticos que si bien logran mantener los valores sanguíneos dentro de los límites deseados con frecuencia lo hacen a expensas de una agresión que importa un fuerte castigo contra el organismo con merma de su capacidad vital

Siguiendo las normas de cuidado general del enfermo y de utilizacion racional de los medios terapéuticos más conocidos con un criterio eminentemente conservador del organismo creemos lograr una mayor vida util de estos enfermos Nuestros leucémicos viven hoy más y mejor En gran parte por el auxilio de los antibioticos que los protegen contra las multiples intercurrencias infecciosas pero también por el mayor conocimiento de esta enfermedad y de sus multiples modalidades evolutivas

Creemos también que los trabajos experimentales sobre el tratamiento de los leucémicos están reservados a las instituciones que cuentan con todos los medios de control Sus conclusiones deberían ser guardadas tan secretamente como las de la bomba atomica hasta tanto sean de real eficacia Sucede corrientemente que los propios enfermos por la información periodística tienen conocimiento de estos ensayos se inquietan con esperanzas caducas gastan ingentes sumas de dinero para conseguir muestras de una medicación que no está en venta y obligan a su médico a probarla con el abandono temporario de aquellas medidas terapéuticas que si bien no tienen un valor curativo permiten un relativo equilibrio

En resumen decimos que en las leucemias tratamos fundamentalmente al enfermo y no a la sangre si bien ésta también nos sirve de guia en el conjunto clínico humoral Y con la fé puesta en que los rápidos progresos de la ciencia revelaran el misterio esperamos vivir la emoción del momento en que se conmute la pena de muerte que aun se cierne sobre estos enfermos

Pathogenesis of Leukemia Deduction on Clinical Basis Our Experience in the Treatment of Leukemic Patients

The clinical observation of numerous cases has led to multiple suggestions related with the pathogenesis of leukemias The authors make special references to the emotional factor as a frequent cause of the breaking out of leucosis They believe that at the present time with the means available leukemic patients attain a greater longevity and a useful life

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The Treatment of Acute Leukemia with 4 Amino Pteroyl Glutamic Acid, Cortisone and ACTH

S L RABASA*

This paper is based on the treatment of 17 patients suffering from acute lymphatic and two from acute myelocytic leukemia One of the latter was a boy 18 years old and the others were children from 9 months up to 11 years old

Aminopterin was given discontinuously in all the cases at a dose of 0 5–1 0 mg a day until a therapeutic or toxic response was apparent Relapses were treated in the same way We got the impression that with this procedure the mean and maximum survival of the patients were approximately the same as with the continuous method of therapy (6 5 and 18 months respectively) while less toxicity seems to occur two severe intoxications in this series The patients with a good response were almost free of toxic symptoms until the end

No patient tolerated more than three courses of aminopterin Tolerance diminished after each treatment in every patient together with a decreased sensibility of the leukemic cells the same dose inducing less effect

The management of the patients was done by daily clinical and hematological examinations Complete recovery induced by aminopterin alone was obtained in 6 out of 14 patients all of them suffering from the lymphatic type

Cortisone and/or ACTH were given in 13 cases With them alone no complete recovery was obtained but all the patients excepting two showed a variable degree of improvement We got the impression that they induce some effect more frequently than aminopterin but without reaching its level of efficiency as regards the degree and duration of recovery It may be concluded from this that hormones are intrinsically less effective than the antifolics but render more constant results as a consequence of their safer and wider dosage range

Small doses of aminopterin seem to have a stimulatory effect upon the bone marrow This phenomenon has been seen 12 times in this series and twice in cancerous patients hematologically normal This fact suggested the convenience of testing a therapeutic procedure based on the use of cortisone first and aminopterin afterwards at a stimulatory dosage Two complete recoveries were obtained by this method in two patients who previously failed to show any response either to cortisone or aminopterin alone This phenomenon can be explained assuming that the recovery takes place in two stages In the first one the bone marrow is cleared of leukemic cells Apparently it can be accomplished with the same efficiency by cortisone or ACTH and by antifolics Either of the two on the other hand may be effective when the other one has failed In the second stage the bone marrow is occupied again by normal medular families This stage is more effectively produced by the antifolics than by hormones The combination of first hormones and afterwards antifolics at a stimulatory dosage may be advisable we believe when hormones induced a good bone marrow depletion of leukemic cells without further normalization Under these circumstances a complete recovery may be obtained by the use of such a low dose of aminopterin as 1 or 2 mg

TRATAMIENTO DE LA LEUCEMIA CON AMINOPTERINA CORTISONA Y ACTH

Se trataron 17 casos de leucemia linfógena en niños y 2 de leucemia mielógena (uno era un joven de 18 años) Se administró 0 5 a 1 mgr de Aminopterina por día hasta obtener respuesta terapéutica o tóxica repitiendo el tratamiento al producirse la recaída Se observaron remisiones completas en 6 de 9 leucemias linfógenas La supervivencia media fué

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de 6 5 meses y la máxima de 18 meses Los síntomas tóxicos son menores con este esquema de tratamiento sólo hubo 2 casos de intoxicación grave y los 6 que respondieron estuvieron casi libres de signos toxicos La tolerancia y la eficacia disminuyen progresivamente al repetirse los tratamientos ningun paciente tolero más de 3 Con cortisona y /o ACTH se obtuvo una remision parcial (nunca completa) en los 13 casos tratados Pequeñas dosis de Aminopterina produjeron un efecto estimulante de la médula ósea en 12 leucémicos y en ? pacientes con cancer pero hematológicamente normales Se combino el tratamiento con cortisona o ACTH y aminopterina en la siguiente forma Se administró cortisona y ACTH hasta obtener el efecto terapéutico luego se dio una pequeña dosis estimulante de aminopterina (1 a 2 mgr) con el objeto de obtener la normalización del cuadro hemático En 2 casos en que no se obtuvo normalizacion con cortisona o aminopterina solas se logró esto con el tratamiento combinado

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Treatment of Chronic Leukosis with Benzene and its Homologues Role of the Methyl Donors

LEON BRAIER*

The lower toxicity of toluene as compared to benzene confirmed by the author in animals encouraged him to use this substance for the treatment of chronic leucosis in man The administration of relatively high doses of toluene (as much as 12 grs per day) during four or more weeks proved to be incapable of modifying the clinical and hematological picture of leukemia It was thought that this inefficacy of the toluene could be related to the presence of a methyl group in its molecule and to the different metabolism of toluene in the organism without formation of oxidation products (quinones etc) that for some authors would be responsible agents of the myelotoxic and therapeutic action attributed to benzene The same patients were subsequently treated with benzene and labile methyl donor substance (choline and methionine) without any clinical and humoral changes while benzene alone even in relatively low doses produced the effects already known a fall in the number of leucocytes increase in the number of red cells and hemoglobin reduction of the spleen general improvement etc It is expected to confirm these results with the study of the benzenemia in patients treated with toluene alone with benzene alone and with benzene associated with methyl donors

Benzene was administered to about ten patients suffering from chronic leukemia (myeloid and lymphoid) with encouraging results in the majority of the cases Attention must be called to the notable tolerance for the drug that nearly all the patients showed even those receiving very high doses (as much as 10 grs per day) during weeks and months

It seems logic to accept that the administration of methyl donors (principally choline and methionine) is capable of annuling the toxic action of benzene by its conversion in toluene or inactive methylbenzene Up to now a similar circumstance had not been mentioned for other therapeutical agents of leukemias such as X rays radioactive phosphorus aminopterin etc

TRATAMIENTO DE LAS LEUCOSIS CRÓNICAS CON BENCENO Y SUS HOMÓLOGOS PAPEL DE LOS DADORES DE METILOS

La menor toxicidad del tolueno frente al benceno comprabada por el autor en animales llevo a éste a ensayar el empleo de aquella sustancia en el tratamiento de las leucosis cronicas

Instituto Municipal de Hematologia y del Ministerio de Salud Publica Buenos Aires Argentina

del hombre La a lministración de dosis relativamente altas de tolueno (hasta 12 g por día durante cuatro semanas o más) se mostró incapaz de modificar el cuadro clínico y hematoló gico de la leucemia Se pensó que esta ineficacia del tolueno podía estar ligada a la presencia de un grupo metilo en su molécula y al diferente metabolismo del tolueno en el organismo sin formación de productos de oxidación (quinonas etc) que serían para algunos autores los responsables de la acción mielotóxica y terapéutica atribuídas al benceno Los mismos pacientes fueron tratados a continuación con benceno y sustancias dadoras de metilos lábiles (colina y metionina) sin cambio alguno de de el punto de vista clínico y humoral mientras que el benceno solo aun en dosis relativamente bajas produjo los efectos ya conoci dos *caída del numero de leucocitos aumento del numero de hematíes y de la hemoglobina* reducción del tamano del bazo mejoría general etc Se espera confirmar estos resultados por el estudio de la bencenemia en pacientes tratados con tolueno solo con benceno sólo o con benceno asociado a dadores de metilo

El benceno fué administrado a unos 10 pacientes con leucemia crónica (mieloide o linfá tica) con resultados alentadores en la mayoría de los cases Cabe destacar la notable toleran cia al medicamento que mostraron casi todos los enfermos tratados incluso aquellos que recibieron dosis muy elevadas (hasta 10 g por día) durante semanas o me es

Parece lógico aceptar que la administración de dadores de metilos (principalmente colina y metionina) sea capaz de anular la accion toxica del benceno por su conversion en tolueno o metilbenceno inactivo Una circunstancia análoga no había sido mencionada hasta el presente para otros agentes terapéuticos de las leucemias tales como los rayos X y el fósforo radioactivo la aminopterina etc

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Radiotherapy in Cases of Leukemia

JOSE LUIS MOLINARI y EMILIO CESAR ROSSELLI*

We believe that in spite of more modern treatments radiotherapy is still the best treat ment of leukemias The treatment may be local or general In acute l ukemias regardle s of th ir type X ray treatment should not be used except occasionally in small doses of 50 r for large adenopathies

Our techniques in general lines are as follow for the local treatment irradiation of the spleen by fields according to its size (anterior posterior and lateral) The same applies for liver The doses are 100 or 200 r once or twice a week 0 5 to 1 Cu filter 40 to 50 cms distance and 6 to 15 Ma The total doses 500 to 800 r occasionally 1000 to 1200 r Sternum and spinal processes take smaller doses For superficial adenopathies we u e 100 to 200 r per session rarely reaching 800 to 1000 r

For total irradiation we use homogeneous doses of 10 to 20 r per session distance 3 60 to 2 mts 0.5 to 1 Cu filter and 200 to 300 Kv once or twice a week alternating dorsal and ventral exposures

With these techniques we have treated 95 cases 65 myelogenous and 28 lymphatic having obtained remissions of more than 13 months

We have kept some patients alive for more than five years in the myelogenous forms and more than 10 years in the lymphatic forms with an average of 2½ years for the first and 3½ years for the last

Facultad de Medicina de Buenos Aires Argentina

Conclusions

(1) We consider x ray the best treatment for leukemia

(2) Other forms of treatment such as nitrogen mustard cortisone radioactive isotopes and blood transfusions are very useful when associated with x ray

(3) We prefer the local treatment although we associate it to the spray irradiation when indicated

(4) We do not use predetermined doses or techniques deciding according to the case

(5) We consider that the x ray series should be spaced as far apart as the patient s condition will allow using in the meantime any of the other methods for treatment mentioned above

(6) X rays should not be given in the acute forms

(7) We follow the patient s general condition and blood counts

(8) We think the ideal situation would be to treat the patient while in the latent period of their disease

Radioterapia en las Leucemias

La razon que nos ha movido a presentar este trabajo es la de dejar establecido que a pesar de los modernos tratamientos preconizados continua siendo la radioterapia sola o asociada el medio más eficaz para tratar las leucemias

La exquisita radiosensibilidad de los leucocitos atacados en su nucleo da lugar despues de una aplicacion de rayos X a una leucopenia acentuada y sucesivas aplicaciones de altas dosis pueden provocar en el animal de experimentación un síndrome hemogénico mortal

Aprovéchase la accion de las radiaciones sobre los órganos linfoides y el tejido mieloide para reducir o hacer desaparecer la metaplasia de esos tejidos tratándose de que la dosis instituída no llegue a producir a causa de la leucolisis reacciones alérgicas tan intensas que ocasionando la desaparicion de los eosinófilos y de las mastzellen conduzcan a la muerte por agotamiento medular

En cuanto a la forma de aplicacion puede ser local o general En las leucemias agudas cualquiera sea su tipo no debe nunca emplearse la roentgenterapia y solo como caso de *excepcion podría utilizarse a pequenas dosis de 50 r cuando gruesas adenopatías* mediastinales produzcan fenómenos asfícticos

Las técnicas que empleamos con algunas salvedades para el tratamiento local son irradiación del bazo con campos de acuerdo a su tamaño anterior posterior o laterales Hígado en la misma forma Dosis 100 a 200 r una o dos veces por semana Filtro 0 5 a 1 Cu distancia 40 a 50 cms Ma 6 a 15 Dosis totales habitualmente 500 a 800 r eventualmente 1000 a 1 200 r Esternón epífisis dosis menores En las formas linfoides cronicas los *ganglios superficiales son irradiados localmente con dosis que varían entre* 100 a 200 r por sesion raramente llegamos a 800 o 1000 r Irradiamos regularmente los ganglios mediastinales y abdominales La tele radioterapia se asocia a la local 10 a 20 r por sesión

En cuanto a la roentgenterapia total se realizará a dosis homogéneas de 10 a 25 r por sesion distancia 3 60 a 2 mts filtro 0 5 a 1 Cu 200 a 300 Kv Sesiones espaciadas una a dos por semana alternando un campo dorsal y uno ventral

Con estas técnicas y sus variaciones hemos tratado 93 casos 65 mieloideas y 28 linfoideas obteniéndose *remisiones de hasta más de 13 meses con roentgenterapia sola*

Hemos obtenido supervivencias de más de 5 anos en formas mieloideas y más de 10 años en formas linfoideas con una supervivencia media de 2½ años en las mieloideas y de 3½ anos en las linfoideas

Conclusiones

(1) Consideramos a la roentgenterapia en la actualidad el tratamiento más eficaz de las leucemias

(2) No parece muy util su asociacion con otros medios como el gas mostaza cortisona isótopos radioactivos (I 32 Na24) transfusiones sanguíneas

(3) Damos preferencia al tratamiento local aunque lo asociamos al general con indicaciones precisas

(4) No usamos una técnica determinada ni dosis preestablecidas cada caso lo indicará

(5) Consideramos que las series deben estar alejadas lo más posible mientras el estado general del enfermo así lo permita En el interín podría utilizarse cualquiera de los medios eficaces ya descriptos si fuera imprescindible

(6) Contraindicamos en forma absoluta la roentgenterapia en los procesos agudos

(7) Vigilamos el estado general del enfermo y especialmente su fórmula hemática

(8) Creemos que lo ideal sería tratar al enfermo en el período de latencia

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Unusual Hematologic Syndromes Associated with Tuberculosis

TIBOR J GREENWALT, BERTRAM H DESSEL and JOSEPH M LUBITZ*

Leukemoid blood pictures are known to be associated with tuberculosis With the advent of new drugs effective against tubercle bacilli it has become increasingly important to distinguish these cases from the leukemias

R T a 65 year old Negro male was admitted with pneumonitis WBC ranged between 1400 and 5800 Differential count was normal on admission but subsequently showed up to 30% myeloblasts and 17% myelocytes Ante mortem bone marrow studies were compatible with a diagnosis of subacute myelocytic leukemia Necropsy revealed widespread tuberculous involvement The bone marrow was compatible with leukemia but there was no leukemic infiltration of other tissues

H H a 33 year old white male was admitted with a diagnosis of acute myeloblastic leukemia WBC was 6 750 with 47% myeloblasts and 6% myelocytes Terminally WBC was 3 900 with 14% myeloblasts and 10% myelocytes The myelogram was compatible with a diagnosis of acute leukemia Autopsy disclosed miliary tuberculosis The bone marrow and testes were infiltrated with blast cells There was no leukemic infiltration in other tissues

Sindromes Hematológicos poco Comunes Asociados con la Tuberculosis

Cuadros sanguíneos con reacciones leucemoides se han observado asociados con la tuberculosis

Con la aparición de nuevas drogas efectivas contra la tuberculosis bacilar se ha hecho de suma importancia saber distinguir estos casos de las leucemias

P T un negro de 64 años fué admitido con pulmonitis Los leucocitos oscilaban entre 1400 y 5800 La fórmula leucocitaria era normal al ser admitido pero posteriormente mostro 30% de mieloblastos y 17% de mielocitos Estudios ante mortem de la médula eran compatibles con el diagnóstico de leucemia mielocítica sub aguda La autopsia reveló una infiltración difusa tuberculosa

La médula era compatible con leucemia pero no se encontró infiltración leucémica en otros tejidos

H H un hombre de 33 anos fué admitido con un diagnóstico de leucemia mieloblástica

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aguda *Leucocitos 6750 con 47% de* mieloblastos y 6% de mielocitos En el período final tenía 3200 leucocitos con 14% de mieloblastos y 10% de mielocitos El mielograma era compatible con el diagnóstico de leucemia aguda La autopsia reveló una tuberculosis miliar La médula y los testículos tenían infiltración de células blásticas No había infiltración leucémica en otros tejidos

La posibilidad de una tuberculosis asociada debe ser descartada en cada caso de leucemia

Cuatro casos de interés serán incluídos en el relato publicado

III communication 14

Regional Statistical Study of Leukemias Hemocytoblastic Monocytoid Leukemia

OSVALDO KESSLER LUDWIG*

The author has studied in the last 10 years 135 cases of leukosis out of [illegible],300 blood counts both from his hematologic private practice and the Porto Alegre General Hospital All counts were done personally by the author These cases are related as to age and sex

Curves are made of their incidence in every 100 000 inhabitants for each decade of life The curves obtained showed different rates from those currently presented by the authors showing that the age incidence for the various types of leukosis are not correct *as is generally* admitted From the analysis of these curves it seems possible to imply the participation of endocrine and metabolic factors as conditioning the different varieties of leukosis

A plea is made to all who are interested in the subject to study larger series of cases according to this concept so it would be possible to conclude that the rates arrived at will stand the trial of concerted efforts

Estudio Estadistico Regional de las Leucemias Leucemia Hemocitoblastica Monocitoide

El autor ha estudiado en los ultimos 10 anos 135 casos de leucosis encontrados en su servicio particular de hematología y en el hospital General de Porto Alegre entre [illegible] 300 hemogramas ejecutados personalmente

Estos casos son relacionados con la edad y el sexo y se han construido curvas en las que para cada década de la vida determina su frecuencia por cada 100 000 habitantes

Se han obtenido así curvas de frecuencia muy diversas de las que habitualmente se admiten por los autores demostrándose que no son exactas las preferencias por edad para los diversos tipos de leucosis que comunmente se admiten

Del análisis de esa curvas parece posible formular hipótesis referentes a factores endocrinos metabólicos como condicionantes de la aparición de las diferentes variedades de leucosis

Se solicita a los interesados en este problema una especial atención y empeño para estudiar con este criterio mayor casuistica y comprobar si se mantienen o no los índices obtenidos

Laboratorio das Clinicas de la Facultade de Medicina Laboratorio Central de Santa Casa de Misericordia Porte Alegre Brazil

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Probable Cellular Location of the Antileukemic Protective Substances

G GASIC, G HOECKER O PIZARRO H MATURANA, E MARDONES*

When the lymphatic cells were eliminated from the system by a short period of therapy with cortisone *immunity to leukemia could not be passively transmitted to an AK strain* of mice but this was possible with C58 mice in spite of using donor spleens from a similar leukemic resistant strain As a result of these findings various interpretations are discussed When this same experiment was done in AK mice before or during the immunizing process or after immunity had been consolidated no changes were found with regard to the development of immunity nor its disappearance once established Protective substances could not be found in the plasma of resistant animals when these were treated for a short period with cortisone With the exception of what was observed in Ak mice the experiments performed do not seem to confirm the lymphatic origin of the substances responsible for anti leukemic protection Nevertheless new experiments must establish the veracity of these conclusions

PROBABLE UBICACION CELULAR DE LAS SUBSTANCIAS PROTECTORAS ANTILEUCEMICAS

Al eliminarse las células del sistema linfático—mediante un corto tratamiento cortisónico la inmunidad no pudo ser trasmitida pasivamente a ratones AK pero sí a ratones C58 a pesar de usarse bazos de dadores que procedían de una misma cepa resistentes a una misma línea leucémica En relación con estos resultados se discuten varias posibilidades de interpretación Cuando se hizo lo mismo en ratones AK antes o durante el proceso de inmunización o después que la inmunidad se había consolidado no se observó un cambio ni en la formación de la inmunidad ni desaparición de ésta una vez establecida Tampoco se evidenciaron sustancias protectoras en el plasma de animales resistentes al ser tratados por un *período corto con cortisona Con la salvedad de lo observado en ratones AK* en un experimento de trasmisión pasiva de la inmunidad los demás resultados no parecen confirmar un origen linfocitario de las sustancias responsables de la protección antileucémica Nuevos experimentos sin embargo deberán establecer si esta conclusión transitoria es válida o no

Instituto de Biología Juan Noe Universidad de Chile Santiago Chile

This work was partly aided by the Rockefeller Foundation

PART IV

Manifestations of Radioactivity on Hematopoietic Organs and Hemostasis

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Repercusión de las Radiaciones sobre los Organos Hematopoyéticos

Radiation Injury and Leukemia among the Japanese Cases Observed in Hiroshima

S AMANO*

FROM OUR observations in Hiroshima the forms of atomic bomb injury are classified in 3 groups namely (I) acute form (II) subacute form and (III) chronic form

The symptoms of the acute form began just after the explosion and persisted for 5–6 days thereafter We have 3 cases of this form that were autopsied 4 days after the bombardment The symptoms of the subacute form, very similar to those of agranulocytosis began after 12 days and were noted to the second month Our 22 case belonging to this form were autopsied 4–6 weeks after the explosion Referring to the chronic form I would like to report here only those confined to leukemia according to the study of Japanese hematologist Dr Yamawaki (April 1952)

General features of the acute and subacute forms of atomic bomb injury may be summarized briefly

I *Acute form* (autopsied 1–5 days after the accident, 3 cases)

Direct changes burn of the skin trauma emphysema of the lung damages in nuclear divisions of various organ cells such as testis hematopoietic and digestive organs

Indirect changes collapse of the cardiovascular system atrophy of endocrine glands

II *Subacute form* (autopsied 4–6 weeks after the accident 22 cases)

Direct changes depilation agranulocytosis panmyelophthisis atrophy of the digestive tracts pluriglandular atrophy

Indirect changes bacteremia gangrenous stomatitis focal pneumonia hemorrhagic and necrotic colitis nephritis collapse of cardiovascular system

In brief the acute form was dominated by traumatic shock while the subacute form was characterized by agranulocytosis and aplastic anemia

I will discuss the histological changes of the spinal bone marrow In their blood picture these cases are aplastic anemia however the changes in the bone marrow were not so simple

Their varieties may be pointed out as follows namely complete panmyelophthisic marrow fibrous marrow partially cellular and partially fatty marrow strongly megakaryocytoplastic marrow

Four weeks after the explosion the external marrows were completely aplastic with strong plasmacellular reaction and a more or less monocytic reaction After that came monocytic marrow with slight erythroblastic reaction The erythrocytic series began to regenerate earlier than the granulocytes And the regeneration of neutrophiles was characterized by toxic granulation of these cells After

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that all sorts of myeloid cells appeared in the bone marrow, but there was a muturation arrest in neutrophiles for some period

These relations may be summarized in the following 4 forms of the bone marrow namely (1) aplastic type (2) hypoplastic type, (3) maturation arrest type and (4) normal type In the severe state, the youngest types of myeloid cells were very sensitive and damaged intensely This peculiarity may also be understood from the karyotoxic nature of the radiation

In some cases the white cell count decreased 2 days after the bombardment to below 1000, while some of them showed after several days, recovery in the leukocyte count and some of them even leukocytosis But there are some other cases which were at first nearly normal in the leukocyte count and showed later agranulocytosis and panmyelophthisis Such cases began to recover after 6 weeks

To make clear such peculiar phenomena we must give attention to the induced radioactivity of P (half life of which is enumerated as 14 days), which is rich in bone tissue and mobilized at the part of the spongious bone

TABLE 1 —*Leukemic Mortality in Hiroshima City Hiroshima Prefecture and Japan (1950) (Yamawaki 1952)*

	Population	Leukemic mortality	Mortality among 10^5 population
A—Bombarded in Hiroshima	98 265	8	8 14
B—Non bombarded in Hiroshima	187 449	5	2 67
C—A + B	285 712	13	4 55
D—Hiroshima prefecture	2 081 865	34	1 65
E—Whole Japan (1949)	82 200 000	1120	1 36

TABLE 2 —*Frequency and Mortality in Leukemia among the Bombarded in Hiroshima Prefecture and the Non bombarded in Hiroshima City (1949-1951) (Yamawaki 1952)*

	Population	Leukemia		Frequency among 10^5 population	Mortality among 10^5 population
		Frequency	Mortality		
Bombarded	125 169	69	35	39 15	27 96
Non bombarded	187 449	19	18	10 14	9 60

TABLE 3 —*Frequency and Mortality of Leukemia among Bombarded People in Hiroshima Referred to the Distances from the Bombing Center (1949-1951) (Yamawaki 1952)*

Distances from the bombing site (M)	Population	Leukemia		Frequency among 10^5 population	Mortality among 10^5 population
		Frequency	Mortality		
0-999	1 400	8	8	571 43	571 43
1000-1499	10 596	18	13	169 88	122 69
1500-1999	19 002	5	3	26 31	15 [illegible]9
2000 and over	67 269	6	6	8 92	8 92
Total	98 265	37	30	37 65	30 53
Non bombarded	187 449	19	18	10 14	9 63

Besides we measured in many cases the induced radioactivity of autoptic tissues with the aid of the Geiger Muller counter In one case without tissue fixation the values of the various organs were as follows

Highest radioactivity was found in bone tissues bone marrow brain and lung with focal pneumonia

Middle radioactivity was found in spleen blood liver large intestine

That radioactive P played a chief role in these data may be easily presumed The brain tissue contains a large quantity of radioactive P but it does not belong to karyoactive tissue

III Chronic form especially with leukemic changes

More than sixteen months after the bombardment leukemic patients of various types appeared among the people who had experienced bombardment in Hiroshima

The curve of this leukemic incidence gave a maximum peak 3–4 years after the accident (Yamawaki cf tables 1 2 3)

From those results we may conclude that the frequent occurrence of leukemia in Hiroshima is coincident with the distribution of the patients who suffered acute and subacute forms of atomic bomb injury Therefore we may assume that the high incidence of leukemia results from exposure to the atomic bomb

It must be also mentioned that some of these leukemic patients had no anamnesis of such symptoms corresponding to acute and subacute forms after the bombardment but experienced the explosion only inside the 2 000 M circle from the bombing center

Lesiones por Radiacion y Leucemia entre los Japoneses Casos Observados en Hiroshima

Las formas patológicas causadas en Hiroshima por la bomba atómica pueden ser clasificadas en tres grupos

I *Formas agudas* (autopsiadas 4 5 días después del bombardeo) que corresponden a las lesiones del shock traumático

II *Formas subagudas* (autopsiadas 4-6 semanas después del bombardeo) que corresponden a las de la agranulocitosis y anemia aplástica con cuatro tipos de médula ósea 1) Tipo aplástico 2) Tipo hipoplástico 3) Tipo de detención de maduración 4) Tipo normal

III *Formas crónicas* particularmente con alteraciones leucémicas Más de 16 meses después del bombardeo se observó en Hiroshima una mayor frecuencia de procesos leucémicos con un máximo a los 3–4 anos de ocurrido el bombardeo Estudiando la distribucion de los pacientes se observó coincidencia con la distribución de los enfermos que sufrieron alteraciones de tipo agudo o subagudo Por tanto debe aceptarse que el aumento de la frecuencia es debido a la exposición a la bomba atómica

IV 2

Modification of Acute and Chronic Irradiation Injury in Experimental Animals by Injection of Bone Marrow and Tissues Originating from Hematopoietic Organs

EGON LORENZ and CHARLES C CONGDON*

THE EXPERIMENTS of Jacobson[1] on modification of the acute irradiation syndrome in mice by spleen protection show that abundant hematopoiesis occurs in the protected spleen a few days after exposure to a lethal dose of x radiation Recovery of the bone marrow follows shortly afterwards It seems tempting to assume that seeding of hematopoietic elements from the spleen may play a role in the recovery process If this be the case seeding with bone marrow cells should also be effective in hastening the recovery process Although numerous reports on the clinical use of a variety of bone marrow preparations in blood dyscrasias exist in the literature during the last fifty years the clinical value has not been proved No reports on the use of bone marrow to modify irradiation injury in man were found Earlier animal experiments[3, 4, 5] were ineffective in modifying irradiation injury by bone marrow injections This failure may have been caused by two factors (1) injection of non viable cells and (2) use of genetically heterogeneous bone marrow Avoiding these factors immediate success was obtained in our first experiments[6]

The animals used in these experiments were mice of various inbred strain and inbred guinea pigs of two families (2 and 13) The 30 day LD_{50} for the mice range from 560 r to 650 r and doses of 800 r and 900 r are 100 per cent lethal in these mice The 30 day LD_{50} of guinea pigs of family 2 is approximately 400 r and for family 13 somewhat higher A dose of 550 r will kill 100 per cent of the animals of the first family and a dose of 750 r will kill all animals of the second family All doses are tissue doses and care was taken that the tissue dose was approximately uniform over the whole animal

The bone marrow suspensions were obtained by aspiration of the marrow of the four long bones of mice (femurs and humeri) into a needle fitting into the marrow cavity and by suspending the marrow in 0 5 cc of buffered saline[7] The amount of bone marrow obtained from a mouse by this method was approximately 1-1 5 mg of wet tissue Guinea pig bone marrow suspensions were also obtained from the four long bones The bones were cracked longitudinally a section of the bone was removed and the marrow lifted out with blunt forceps Approximately 100 mg of marrow was thus obtained from one guinea pig It

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TABLE 1.—*Effects on Survival of Homologous Bone Marrow Injection following Acute Irradiation*

Exp	Dose	Treatment	Strain	Number Animals	Number Dying on Indicated Number of Days: 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16–21	Mortality (Per Cent)
1	700	None	I	39						5	1	9	4	4	4	3	3			2	90
2	900	None	LAF	38					2	6	4	5	9	6	4					2	100
3	900	II	L	20						2	2	2	2								40
4	900	IV	I	20									2	1					1	2	30
5	900	II	LAF₁	16				1		1		2									25
6	900	IV	LAF₁	30				1		1		2		1							17
7	900	None	C₃H_b	60				2	4	9	20	8	8	3	6						100
8	900	II	C₃H_b	63			7	5	9	8	5	7	7		2	2	2	3			90
9	900	IV	C₃H_b	56					2	6	6	2								2	32
10	900	None	A	[illegible]			2	12	6	1		1									100
11	900	II	A	19								1	8	4	2					1	84
12	900	IV	A	20																	0
13	900	IV	A	16			1							2							19
14	500	None	CF Fa2	11									2	3	2	2			1		91
15	500	II	CF Fa2	6									1	1	1						50
16	[illegible]	IV	GF Fa	5										[illegible]							40
17	[illegible]	IV	GF Fa2	14										1	1						14
18	700	None	GF Fa13	12								1	6	1	5						100
19	700	IV	GF Fa13	21																	0

* Last died on 25th day

was treated in a similar manner to the mouse bone marrow and suspended in 0.5 cc of buffered saline. Injections were made in mice either intravenously into the tail vein or intraperitoneally, in guinea pigs intravenously into the saphenous vein, intraperitoneally or intracardially.

The effects of homologous bone marrow injections on survival of genetically homogeneous mice and guinea pigs are shown in Table 1. A survival period of 21 days was selected because by that time full recovery of the hematopoietic tissues had taken place in the bone marrow injected animals. Only occasionally did animals die after 21 days. The cause of their death was obscure, but it was not caused by exhaustion of hematopoietic tissues. Usually, however, the experimental animals were not sacrificed until 30 to 60 days after exposure.

Table 1 shows that intravenous bone marrow injections gave excellent protection in the different strains of mice and guinea pigs: the mortality was reduced from 100 per cent to 0 to 30 per cent. Intraperitoneal injection of homologous bone marrow gave good protection in two strains of mice, but only slight protection in the two other strains. The reason for this has not been determined.

The hematologic studies of irradiated mice give an indication why intraperitoneal injection of bone marrow might be less effective than intravenous injection. The effect of the irradiation on the erythrocyte count in control and injected mice is as follows: The controls are dead before the effect of the irradiation on the erythrocytes is established. The mice injected intravenously show only a transient slight depression, while in the intraperitoneally injected animals recovery of the erythrocyte count does not begin until the fifteenth day. The appearance of reticulocytes in the circulating blood lags behind also in the intraperitoneally injected animals. This lag in recovery is most pronounced in the picture of the circulating leukocytes (fig. 1). Hence animals will still die during this delayed recovery of the hematopoietic system following intraperitoneal injections. In mice in which survival is similar for intraperitoneal and intravenous bone marrow injections this delay in recovery is much less pronounced.

In guinea pigs of family 2, as shown in table 1, decreased mortality ranging from 10 per cent to 50 per cent was obtained, intracardial injection giving the best protection and intraperitoneal injection the least. In family 13, 100 per cent survival was observed following intravenous injection.

The intravenous bone marrow injection following a lethal dose of irradiation modifies strikingly in these animals the well known hematologic effects of irradiation, as can be demonstrated in figures 2 to 5. Figure 2 gives the curve for the hematocrit of irradiated control and bone marrow injected animals. The control animals all die within 11 days with a lethal anemia, thrombocytopenia and leukopenia. No anemia is apparent in the bone marrow injected animals. However, all other blood cells are affected. There is a decrease in the number of cells to the sixth and eighth day parallel to that of the controls. In the bone marrow injected animals rapid recovery sets in thereafter, while in the animals not injected with bone marrow the cells continue to decrease until death. The curve of figure 3 gives the changes in the platelet count, figure 4 those of the leucocyte

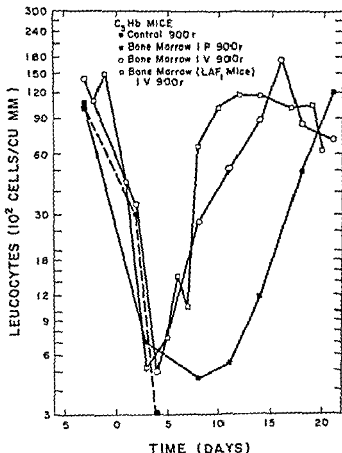


Fig 1—*Leukocyte count of untreated and bone marrow injected mice following a lethal* dose of x irradiation

count and figure 5 the rapid increase in the percentage of reticulocytes after the sixth day

In the data presented thus far the homologous bone marrow injections were given within one half hour after irradiation and freshly prepared bone marrow was administered It seemed of interest to find out what time can elapse between irradiation and injection and still get survival from a lethal dose of irradiation and how long bone marrow emulsion will stay effective before being injected A series of experiments were performed to test the effect of delayed bone marrow *injection on survival Results are given in table 2 Mice of strain A were used in* these experiments the dose was 900 r which is 100 per cent lethal in 21 days The protective effect is excellent in the mice which were given bone marrow 4 32 and 48 hours following irradiation After 72 hours some protection is still obtained The high mortality of 75 per cent in the mice injected 24 hours after irradiation is obscure Figure 6 shows the recovery of the white cell count when the bone marrow was injected 0 1 2 and 3 days after irradiation All animals

was treated in a similar manner to the mouse bone marrow and suspended in 0.5 cc of buffered saline. Injections were made in mice either intravenously into the tail vein or intraperitoneally, in guinea pigs, intravenously into the saphenous vein, intraperitoneally or intracardially.

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FIG 4—Leuko ȝte count of untreated and bone marrow injected guinea pigs of family 13 following a lethal dose of x irradiation

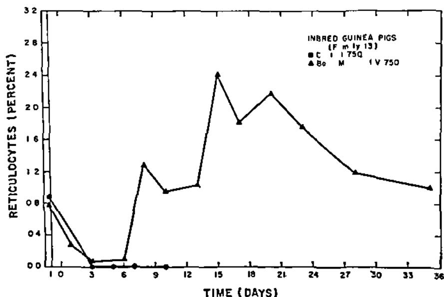


FIG 5—Per cent reticulocytes of untreated and bone marrow injected guinea pigs of family 13 following a lethal do e of x irradiation

FIG 2—Hematocrit of untreated and bone marrow injected guinea pigs of family 13 following a lethal dose of x irradiation

FIG 3—Platelet count of untreated and bone marrow injected guinea pigs of family 13 following a lethal dose of x irradiation

LEUCOCYTES (10^2 CELLS/CU MM)

TIME (DAYS)

INBRED GUINEA PIGS

Fig 4 —Leukocyte count of untreated and bone marrow injected guinea pigs of family 13 following a lethal dose of x irradiation

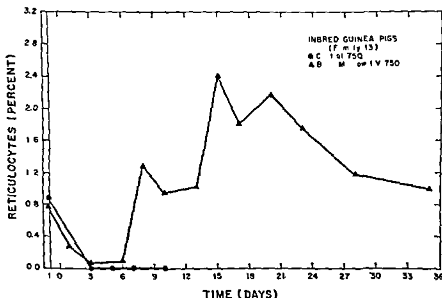


Fig 5 —Per cent reticulocytes of untreated and bone marrow injected guinea pigs of family 13 following a lethal dose of x irradiation

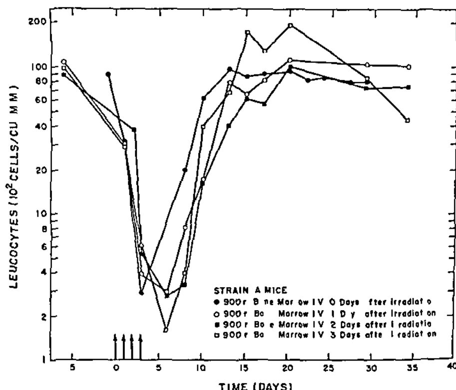


Fig 6 —Recovery of leukocyte count in strain A mice injected intravenously with bone marrow 4 24 36 and 72 hours after a lethal dose of x radiation

were irradiated at zero day the vertical arrows indicate the time of injection The delay of recovery of the leukocyte count in dependence upon time of injection is apparent Table 2 shows in addition that strain A mice bone marrow will protect if the amount of bone marrow injected is decreased to approximately 0 5 mg and if it is aged at room temperature for four hours In a pilot experiment in which guinea pig bone marrow suspension was aged at ice box temperature for 24 or 48 hours respectively good protection was also obtained

All experiments discussed so far deal with the modification of the acute irradiation syndrome It seemed important to learn whether or not irradiation induced chronic anemia could also be influenced by bone marrow injections It was shown in a previous experiment that the majority of guinea pigs exposed daily for 8 hours to 8 8 r of gamma radiation until their red cell count had decreased to about 2 5 million died of anemia soon afterwards or recovered temporarily only to die of a recurrent anemia within 6 months[8] Subsequently a number of guinea pigs were exposed under identical conditions and were given intravenous bone marrow injection at the time of removal from the exposure field Table 3 gives the survival data 79 per cent of the first group (not injected with bone marrow) died while only 18 per cent of the second group (injected with bone marrow) succumbed during a period of 26 weeks The effect of the bone marrow injection is most strikingly shown in the picture of the circulating

TABLE 2.—*Effects of Time Factors in Modification of Irradiation Injury with Intravenous Bone Marrow Injection in Strain A Mice Exposed to 900 r*

Exp	Amount Injected (per mouse)	Time after Irradiation	Number Animals	Number Dying in Indicated Number of Days: 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16–21	Mortality (Per Cent)
1	None	—	22			2	12	6	1		1									100
2	1 mg	10 min	20																	0
3	1 mg	10 min	16			1							2							19
4	1 mg	4 hr	20																	0
5	1 mg	24 hr	16				1	1	6	2		1				1				75
6	1 mg	32 hr	20																	0
7	1 mg	48 hr	16						1			1								12
8	1 mg	72 hr	15						3	2	2	2	1		1					73
9	1 mg	10 min	16				1	1	1										1	25
10	0.5 mg	10 min	20								1	1								10

Bone marrow aged 4 hours before injection

blood In figures 7 to 10 the blood data are given of those animals of the first group which died without or with only little recovery those of the first group which recovered and those which survived after bone marrow injections The *pronounced effect of the bone marrow injections on the return of the blood* picture to normal in comparison to non treated controls is striking especially in the hematocrit the platelet count and the reticulocyte count

Finally it could be shown in a pilot experiment that bone marrow injections also protect against the lethal effects of internally given radioactive substances When radon in equilibrium with its short lived decay products was injected intravenously into mice the dose being 0.017 millicuries per gram 93 per cent died within 30 days For a dose of 0.018 millicuries per gram followed by intravenous bone marrow injections only 67 per cent died within the 30 day period

From these blood data as well as those of the acutely exposed guinea pigs it seems that the injected erythropoietic cells may play the major role in the modification of irradiation injury in guinea pigs This does not exclude however the fact that other hematopoietic tissues as well as tissues originating from hematopoietic organs may also play a role in the recovery process A series of experiments were performed testing such suspensions for their effectiveness in the modification of irradiation injury These are listed in table 4 Groups of two different homogeneous hybrids (BAD and LAF_1) and one inbred strain (A) were

TABLE 3.—*Survival of Guinea Pigs Exposed Chronically to a Limited Dose of Gamma Radiation with and without Subsequent Injection of Bone Marrow*

Exp	Number Animals	Treatment	RBC At Time Of Removal × 10⁶	Number Dying in Indicated Number of Week: 1	2	3	4	5	6	7	8	9	10	11	12	13–26	Mortality (Per Cent)
1	48	None	2.58 ± 0.5	11	3	4	3	3	1	2	1		1		2	7	79
2	17	BM IV	2.06 ± 0.[illegible]	1		1								1			18

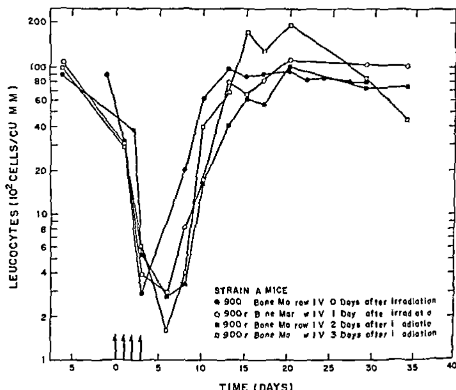


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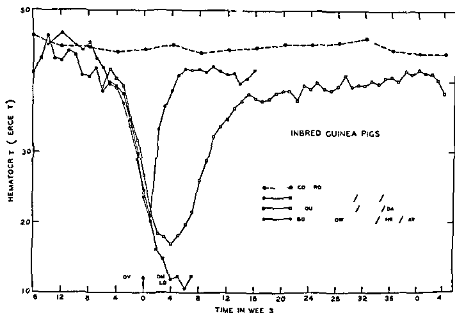


Fig 7 — Hematocrit of chronically exposed guinea pigs and without bone marrow injection

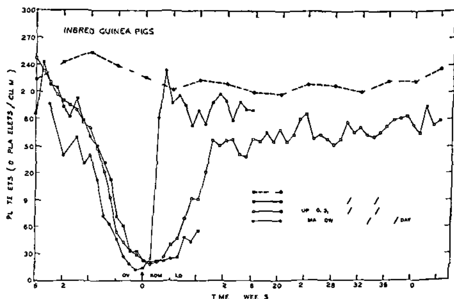


Fig 8 — Platelet count of chronically exposed guinea pigs with and without bone marrow injection

used All animals were exposed to a dose of 900 r which is 100 per cent lethal within 21 days in untreated controls Adult spleen injected intravenously gave good protection Immature spleen or spleen stimulated by irradiating the donor animal and protecting the surgically mobilized spleen by lead and injected three days after irradiation of the donor gave better protection than the adult spleen

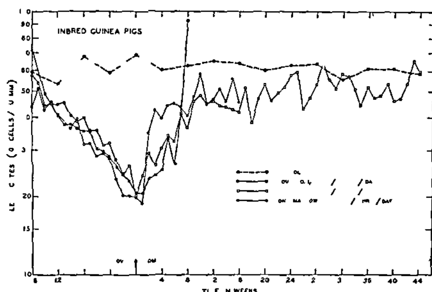


FIG 9—Leukocyte count of chronically exposed guinea pigs with and without bone marrow injection

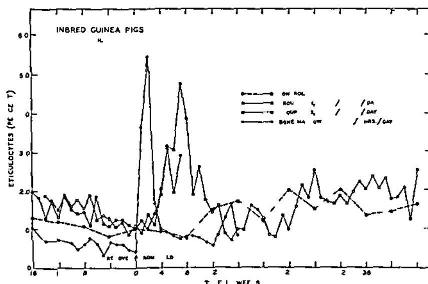


FIG 10—Reticulocyte count of chronically exposed guinea pigs with and without bone marrow injection

This is not surprising because immature mouse spleen shows considerable hematopoiesis in comparison to adult spleen and because in the spleen protected irradiated animals abundant erythropoiesis is mainly observed in the protected spleen

Surprising is the protection obtained by intravenous injection of a reticulum

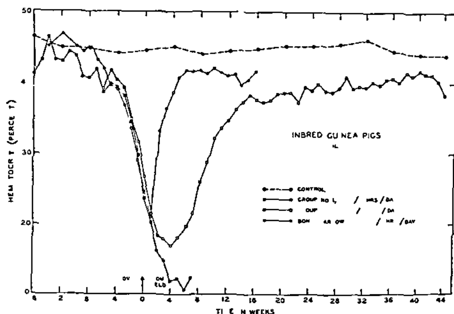


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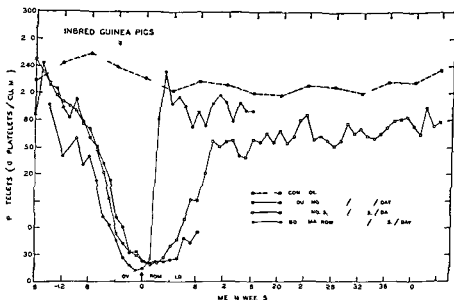


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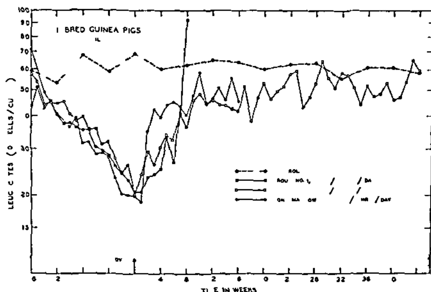


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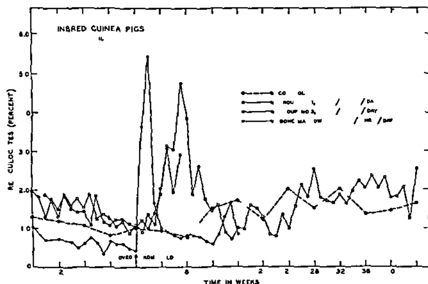


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This is not surprising because immature mouse spleen shows considerable hematopoiesis in comparison to adult spleen and because in the spleen protected irradiated animals abundant erythropoiesis is mainly observed in the protected spleen

Surprising is the protection obtained by intravenous injection of a reticulum

cell sarcoma This sarcoma grows slowly at the site of the implant but metasta sizes in the later stages mainly to the liver The metastatic liver was used for th preparation of the suspension to be injected Normal liver suspension as could b shown gives no protection As the reticulum cells of this sarcoma resembl closely normal reticulum cells, one may speculate that reticulum cells play a important role in recovery from irradiation injury Other tumors such as mouse lymphosarcoma or a guinea pig leukemia (not listed in the table) give n protection

Suspension of thymus of immature mice gave protection in one experimen when injected intravenously In spite of all precautions in the preparation of th suspension, many experimental animals died of embolism shortly or within a fev days after injection Therefore thymic tissue was implanted intraperitoneall following irradiation All animals died within the 21 day period, however, deat was somewhat delayed in comparison to non treated animals

Finally spleens of immature mice were grown in tissue culture the tissu culture fluid was injected into one group of mice of the same strain and th tissue culture cells were injected into another group following irradiation Non of the animals survived the 21 day period but there seemed to be delayed deat in the group injected with the cells The failure of the tissue culture cells to protect may have been caused entirely by injection of an insufficient number o cells Experiments with guinea pig bone marrow tissue cultures injected int guinea pigs intraperitoneally have so far given inconclusive results

This is the present status of our experiments dealing with modification of lethal irradiation injury by injection of homologous tissues It might be well at this point to discuss the effects of such injections on the recovery of the hema topoietic tissues of the irradiated host Independent of the tissue injected, the histologic picture is identical if these tissues were effective in modifying the lethal irradiation injury For purpose of illustration only the histologic data of the recovery process will be discussed which were obtained with homologous bone marrow injections

The typical histologic picture of the hematopoietic tissues of mice following total body exposure to a lethal dose of irradiation was characterized by the following findings In mice exposed to 900 r and killed serially for five days hematopoietic elements showed nearly total depletion by the second day with a few megakaryocytes persisting to the fifth day Depletion of lymphocytes reached its peak on the second and third day Slight return of lymphocytic elements was evident on the fourth day The same observations were made in animals which died 4 to 11 days after exposure In addition in these mice bacteremia was a frequent finding Serial killing of irradiated mice receiving intravenous bone marrow showed that return of hematopoietic cells in the red pulp of the spleen was taking place at three days and was marked by the fifth day In the sternal bone marrow hematopoiesis began approximately the fifth day and was marked by the seventh day and bacteremia was absent

The histologic picture of the hematopoietic tissues of guinea pigs following irradiation with or without bone marrow injections is similar to that of mice

TABLE 4.—*Effects on Recovery of Injection of Tissues Originating from Hematopoietic Organs*

Exp.	Dose	Treatment	Strain	No. of Animals	Number Dying on Indicated Number of Day															Mortality (Per Cent)
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15–21	
1	900	None	LAF_1	35					2	6	4	5	9	6	4				2	100
2	900	IV rad spl*	LAF_1	9						1	1			1	1					44
3	900	IV rad spl	LAF_1	16				1			3	1								31
4	900	IV ½ spl	LAF_1	10											1	1				20
5	900	None	BAD†	64					1	1	4	6	14	14	16	6	2			100
6	900	IV Ret Cell Sa	BAD†	10						1	1	2	3						3	100
7	900	IV Ret Cell Sa	BAD†	15					3	2						1		1		47
8	900	None	A	22			2	12	6	1		1								100
9	900	IV Lymphoma	A	16				1		1	3	7	4							100
10	900	IV Thymus	A	6	1		1				1									50
11	900	IV Thymus	A	10		1	1	1	1	1	4		1							100
12	900	IP Thymus	LAF_1	20						1	1	1	1	8	2	5	1			100
13	900	IV Spl cult fluid	A	10						5		3	2							100
14	900	IV Spl cult	A	20						1	5	2	7	4		1				100

* Spleen stimulated by spleen protection 3 days prior to injection.
† F_1 homogeneous hybrid of C_{57} black and DBA cross.

However, histologic evidence of bacteremia was lacking in the animals given bone marrow injection following an acute lethal dose of irradiation Regeneration of hematopoietic tissue is delayed one to two days in comparison to mice and parallels the recovery of the circulating blood As in mice the recovery of the hematopoietic tissues was slower following intraperitoneal injection

In the following figures (figs 11 to 22) microphotographs are presented which illustrate the effects of intraperitoneal bone marrow injection on the bone marrow of irradiated guinea pigs in comparison to non treated irradiated guinea pigs

Normal sternal bone marrow is shown in figure 11

Figures 12 and 13 show the picture of irradiated animals about the fifth day after irradiation, with and without bone marrow injection The destruction of bone marrow is the same for both animals

Figures 14 and 15 compare the bone marrow on the eleventh and twelfth day Recovery is nearly complete in the treated animal (fig 14) but no recovery is apparent in the untreated animal which died on the eleventh day

Recovery of the hematopoietic tissues of guinea pigs injected intravenously and intracardially is similar except that it is faster in comparison to intra peritoneally injected animals Figures 16 17 and 18 compare intraperitoneal and intravenous injection on the ninth day after irradiation It is interesting to follow the growth of bone marrow elements which developed in the greater omentum of guinea pigs after intraperitoneal injection This is shown in figures 19 to 22

Figure 19 shows the intraperitoneal transplant on the day of irradiation The bone marrow is approximately normal Figure 20 shows the transplant on the third day Note the area of central necrosis with a zone of hemorrhage around the necrotic area At the margin there are still living cells Figure 21 shows the edge of a transplant on the sixth day Nearly all of the transplant is replaced by trabecular bone On the fifty ninth day (fig 22) there is intense blood formation in the marrow spaces between trabeculae

It is evident from the data shown that the fate of the intravenously injected bone marrow is not known It may be assumed that the injected cells passed through the lungs—no evidence was found of bone marrow deposited in the lungs except for a few large particles composed of bone spicules and fat cells—and lodged elsewhere possibly in the bone marrow spleen and perhaps lymph nodes If this be the case it would be nearly impossible to detect the cells after irradiation and impossible to state that the hematopoietic recovery observed in spleen and bone marrow is caused by proliferation of the injected cells The observation on intraperitoneally injected bone marrow which grows luxuriantly in the omentum of the irradiated animals does not seem to be of importance from the point of view of recovery of the host Although gross hematopoietic cellularity of these transplants parallels that of the regenerating sternal bone marrow recovery was delayed in comparison to the intravenously injected bone marrow If we want to assume that seeding caused the recovery after intra peritoneal injection we have to assume in this case also that the seeded cells



Fig 11 —Sternal bone marrow of a normal guinea pig Hematoxylin and eosin 210×

Fig 12 —Depleted sternal bone marrow 5 days after 500 r and intraperitoneal bone marrow injection Hematoxylin and eosin 350×

Fig 13 —Depleted sternal bone marrow 6 days after 500 r Hematoxylin and eosin 350×

Fig 14 —Regenerating sternal bone marrow 12 days after 500 r and intraperitoneal bone marrow injection Hematoxylin and eosin 350×

Fig 15 —Depleted sternal bone marrow 11 days after 500 r Hematoxylin and eosin 350×

However histologic evidence of bacteremia was lacking in the animals given bone marrow injection following an acute lethal dose of irradiation Regeneration of hematopoietic tissue is delayed one to two days in comparison to mice and parallels the recovery of the circulating blood As in mice the recovery of the hematopoietic tissues was slower following intraperitoneal injection

In the following figures (figs 11 to 22) microphotographs are presented which illustrate the effects of intraperitoneal bone marrow injection on the bone marrow of irradiated guinea pigs in comparison to non treated irradiated guinea pigs

Normal sternal bone marrow is shown in figure 11

Figures 12 and 13 show the picture of irradiated animals about the fifth day after irradiation, with and without bone marrow injection The destruction of bone marrow is the same for both animals

Figures 14 and 15 compare the bone marrow on the eleventh and twelfth day Recovery is nearly complete in the treated animal (fig 14) but no recovery is apparent in the untreated animal which died on the eleventh day

Recovery of the hematopoietic tissues of guinea pigs injected intravenously and intracardially is similar except that it is faster in comparison to intra peritoneally injected animals Figures 16 17 and 18 compare intraperitoneal and intravenous injection on the ninth day after irradiation It is interesting to follow the growth of bone marrow elements which developed in the greater omentum of guinea pigs after intraperitoneal injection This is shown in figures 19 to 22

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Fig 19 —Essentially normal bone marrow recovered from the peritoneal cavity a few hours after injection into an irradiated guinea pig Hematoxylin and eosin 350×

Fig 20 —Necrotic mass of bone marrow recovered from the peritoneal cavity 3 days after injection into an irradiated guinea pig Hematoxylin and eosin 30×

Fig 21 —Organized mass of trabecular bone with marrow spaces recovered from the peritoneal cavity 6 days after injection into an irradiated guinea pig Hematoxylin and eosin 19×

Fig 22 —Mature bone marrow transplant after 50 days in peritoneal cavity of irradiated guinea pig Hematoxylin and eosin 30×





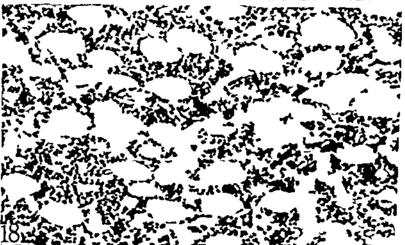


Fig 16 —Depleted sternal bone marrow 9 days after 550 r Hematoxylin and eosin 350×

Fig 17 —Isolated patch of regenerating bone marrow 9 days after 550 r and intraperitoneal injection of bone marrow Hematoxylin and eosin 210×

Fig 18 —Diffuse regeneration of sternal bone marrow 9 days after 550 r and intravenous injection of bone marrow Hematoxylin and eosin 210×

TABLE 5.—*Effects of Intravenous Heterologous Bone Marrow Injections on Survival Following Irradiation*

Exp	Dose	Donor	Recipient (strain)	Number Animals	Number Dying on Indicated Number of Days															Mortality (Per Cent)
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15–21	
1	900	None	LAF_1	35					2	6	4	5	9	6	4				2	100
2	900	GP Fa 2	LAF_1	24							2	1	6	7	4	4				100
3	900	GP Fa 2	LAF_1	10								1	1	4						60
4	900	GP Fa 13	LAF_1	29					3	3	2	4	7	7	2	1				100
5	900	Dog	LAF_1	40					13	8	3	4	6	5		1				100
6	900	Rabbit	LAF_1	18							2		9	5	2					100
7	800	None	LAF_1	20						2	1	2	4	10				1		100
8	800	GP Fa 13	LAF_1	10								1	1	1	1					40
9	800	GP Fa 13	LAF_1	10								1	1	3	1	3		1		100
10	900	None	C_3H_b	60				2	4	9	20	8	8	3	6					100
11	900	LAF_1	C_3H_b	18					4	1		2	3		1					61
12	550	None	GP Fa 2	11									2	3	2	2			1	91
13	500	GP Fa 13	GP Fa 2	4																0
14	450	None	GP Fa 13	12								1	5	1	5					100
15	750	GP Alb	GP Fa 13	5									1		1				1	60
16	750	Rabbit	GP Fa 13	6								4		1	1					100
17	700	Rabbit	GP Fa 13	5								1	2		1	1				100

Last animal died on the 25th day

went from the transplant to bone marrow and spleen. Thus the problem whether the recovery is caused by seeding bone marrow and spleen of the irradiated host with homologous cells which grow luxuriantly and replace the destroyed bone or whether it is caused by a humoral factor produced by the injected cells which stimulates the regrowth of the damaged bone marrow is at this stage undecided. The hypothesis that a humoral factor is involved seems from the foregoing more plausible considering that other tissues such as a reticulum cell sarcoma and perhaps the thymus were also effective in giving protection. This hypothesis would gain in strength if it could be shown that injections of heterologous bone marrow will also modify the lethal irradiation injury. We define a heterologous transfer of bone marrow as one in which the transfer is made between two different highly inbred strains or hybrids of the same species or between animals of different species. A series of experiments was performed and the data are given in table 5.

Heterologous bone marrow within the same species gives good protection. This is shown by the 49 per cent survival of C_3H_1 mice when given approximately 1 mg. of bone marrow of LAF$_1$ mice and as shown by a 100 per cent survival of inbred guinea pigs of family 2 when they were injected with approximately 100 mg. guinea pig bone marrow of family 13 or a 40 per cent survival when injected with the same amount of hybrid guinea pig bone marrow. The number of guinea pigs in these experiments was small on account of lack of inbred animals; nevertheless the results are significant. Bone marrow of guinea pigs injected into mice gave sporadic results. In two experiments good survival was obtained, while in three others all injected animals died. Neither dog nor rabbit bone marrow gave protection, nor did rabbit bone marrow in inbred guinea pigs. In some of these experiments in which the mortality was 100 per cent delayed death was noted. There was also suggestive histologic evidence in some mice which were found dead that partial recovery of hematopoietic tissue was present with death apparently due to bacteremia. When mice were given guinea pig bone marrow intraperitoneally the bone marrow underwent necrosis and partial reorganization of the necrotic mass. There was suggestive histologic evidence that some of the guinea pig bone marrow cells survived several days in the peritoneum of irradiated mice. Finally the experiments of *Jacobson* should be mentioned; he showed that immature mouse spleens transplanted into the abdominal cavity of rabbits gave enhanced bone marrow recovery in comparison to non treated irradiated controls.[9]

What explanation can be given that injections of guinea pig bone marrow into mice following a 100 per cent lethal exposure to radiation gave good protection in two experiments and none in others? Above all it cannot be assumed that in the successful experiment seeding with hematopoietic tissue occurred which resulted in replacing the damaged bone marrow. It seems plausible however that a humoral factor is involved which stimulates the damaged bone marrow to new growth. The amount of this factor produced by the living cells may be very small, but as in homologous bone marrow injections the injected cells stay alive as shown in the intraperitoneal transplants. This factor may be produced

TABLE 5 —*Effects of Intravenous Heterologous Bone Marrow Injections on Survival Following Irradiation*

Exp	Dose	Donor	Recipient (strain)	Number Animals	Number Dying on Indicated Number of Day															Mortality (Per Cent)
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15–21	
1	900	None	LAF_1	38					2	6	4	5	9	6	4				2	100
2	900	GI Fa 2	LAF_1	21							2	1	6	7	4	4				100
3	900	GI Fa 2	LAF_1	10								1	1	4						60
4	900	GI Fa 13	LAF_1	20					3	3	2	4	7	7	2	1				100
5	900	Dog	LAF_1	40					13	8	3	4	6	5		1				100
6	900	Rabbit	LAF_1	18							2		9	5	2					100
7	800	None	LAF_1	20						2	1	2	4	10				1		100
8	800	GI Fa 13	LAF_1	10								1	1	1	1					40
9	800	GI Fa 13	LAF_1	10								1	1	3	1	3		1		100
10	900	None	C_3H_b	60				2	4	9	20	8	8	3	6					100
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12	500	None	GP Fa 2	11									2	3	2	2			1	91
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17	700	Rabbit	GP Fa 13	5								1	2		1	1				100

Last animal died on the 25th day

constantly during the recovery period In injections of heterologous bone marrow the transplanted cells stay alive for several days but production of the humoral factor will decrease as the heterologous cells die It is probably pure chance that in the successful experiments a sufficient number of cells may have stayed alive longer than in the unsuccessful experiments, thus producing the unknown factor sufficiently long enough to aid in the recovery It is known that in the transplantation of heterologous tumor tissue some such tumors will grow if only a sufficient number of animals are used Relatively little is known of the conditions under which such tumors will take Another additive factor may be that bone marrow from some animals is more viable than from others but we do not know yet what factors influence the viability of the bone marrow

Attempts to isolate this factor from bone marrow have failed so far We do not intend to go into detail about the procedures mentioned which consisted in breaking up the cells by sonic vibration or in a Potter grinder by quick freezing and thawing or by lyophilization The reason for the failure may be that relatively large amounts much larger than we have used so far may be needed Our experiments seem to indicate that the continuous presence of this factor will give the best survival Even if we should have isolated the factor by the above procedures it may have been destroyed soon after injection

At present the application of these experiments to clinical medicine is not apparent Total body irradiation has limited application in human disease The possibility of raising the dose in total body exposure is now hypothetically at least visualized It has been suggested that bone marrow injection could be used to increase the amount of nitrogen mustard in the treatment of human leukemia It would be possible also to increase the amount of radioactive isotopes e g I^{131} in the treatment of thyroid cancer and thus prevent the limiting factor of isotope therapy the depression and destruction of hematopoietic tissue There is ample evidence in the literature that bone marrow transfusion can be made in man with no serious ill effects Finally bone marrow injections may be of value in accidental total body exposures to ionizing radiations in the lethal dose range No other effective agents are available

The experiments described were started somewhat over a year ago Many data have been accumulated which throw some light on the fundamental process of irradiation injury The isolation and identification of the hypothetical factor which modifies the injury would be an important step in our understanding of this fundamental process

Modificacion de las Lesiones Agudas y Crónicas por Irradiacion en Animales de Experimentación por Inyecciones de Tejidos Hematopoyéticos

Una dosis letal aguda de 900 r Rayos X fué dada a ratones de los grupos A I C Hb y genéticamente híbridos homogéneos LAF Las inyecciones de suspensiones homologas de médula ósea en buffers salinos ya sea poco después de la irradiación o hasta 2 días más tarde dió un porcentaje de supervivencia de 21 días que oscilaba de 70 a 100 El envejecimiento de la suspensión de médula ósea de una a cuatro horas antes de la inyección dió porcentajes similares de supervivencia como se probó en ratones del grupo A La inyección intraperitoneal de médula osea homóloga en ratones también dió protección pero en grado menor Asimismo cobayos consanguíneos expuestos a una dosis letal aguda de [illegible] r (de

tuvieron una buena protección con inyecciones de su pension de médula osea homologa ya sea por vía intravenosa intracardíaca o intraperitoneal Cobayos expuestos crónicamente a 8 8 r diarios (8 horas) y alejados de la exposición cuando el numero de globulos rojos había descendido a 2 millones murieron de una anemia terminal algunas semanas después de terminada la irradiacion La dosis total acumulada alcanzó aproximadamente a 1000 r Excelentes mejorías se obtuvieron en cobayos expuestos en idénticas condiciones cuando se les inyectó por vía intravenosa suspensiones homologas de médula osea después de terminada la irradiación

El mecanismo de la mejoría no parece ser de origen celular sino más bien parece ser causado por un factor humoral producido por la médula osea inyectada al obtenerse una proteccion cuando médula osea heterologa de un diferente grupo de ratones o cobayos es inyectada por vía intravenosa en ratones agudamente irradiados Los informes obtenidos hasta ahora parecen indicar que varios factores independientes pueden ser responsables de la mejoría ya que la inyeccion intravenosa de tejido tímico o tejido tumoral proveniente de organos hematopoyéticos parece favorecer la mejoría Se presentarán cuadros con datos de supervivencia y microfotografías que ilustran el proceso de mejoría

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IV 3

Disturbances in the Hemostatic Mechanisms Produced by Whole Body Irradiation

E. P. CRONKITE,* GEORGE BRECHER† and L. M. TOCANTINS‡

FOR MANY years generalized purpura and massive local hemorrhages have been recognized as important causes of death from lethal doses of total body radiation.

In 1922 Lacassagne et al.,[1] *Cramer et al.*[2] *and Fabricius Moller*[3] *correlated* the bleeding tendency with a thrombopenia. The observations of Fabricius Moller are especially pertinent. The bleeding tendency in guinea pigs paralleled the depletion of platelets. If the legs were shielded with lead the depression of platelets was prevented and there was no bleeding.

In 1918 a new concept of radiation hemorrhage was introduced by Allen et al.[4] His group believed that "heparinemia" was the primary cause and that thrombocytopenia was a secondary contributing factor. This concept was of particular interest because of the potentialities for definitive therapy through the use of antiheparin agents. The concept of heparinemia has been disputed by others[5-9] and is apparently now satisfactorily eliminated as a cause in the dog.[10]

Lawrence and Valentine[11] observed that cross circulation stopped the bleeding *in the irradiated thrombopenic cat. Brecher and Cronkite*[12] *demonstrated that* parabiosis prevents bleeding in the irradiated parabiont. The prevention of bleeding by these two procedures might be explained by deficiency of a plasma factor, neutralization of an anticoagulant, or increasing the supply of platelets. These possibilities were approached by direct experiment as follows:

(a) Systematic assay of known plasma factors demonstrated that there was no deficiency of prothrombin, accelerator globulin,[13] serum prothrombin conversion accelerator,[14] antihemophilic factor[15] or fibrinogen.[10]

(b) Anticoagulants of heparin-like type were eliminated as an important cause.[10]

(c) The role of the platelet was investigated by in vitro and in vivo studies.

The blood coagulation defect that is produced in the irradiated dog is well correlated with the platelet deficiency. The residual prothrombin after 60 minutes at 37 C progressively increases as the platelet count decreases. The whole blood clotting time increases after the platelets fall below 50 000 per cu mm.[13] Prothrombin utilization is corrected in vitro by the addition of washed platelets to a final concentration of 20 000 per cu mm.

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The development of a method for the separation of platelets on a large scale by Dillard et al [16] made it possible to evaluate the effect of separated platelets on the hemorrhagic tendency Since washed separated platelets are removed from the circulation platelet suspensions in plasma were used Platelets approximately equal in number to the normal circulating mass of platelets were transfused daily in 20 to 40 ml of plasma Each experiment was controlled by a dog transfused by an equal volume of platelet free plasma

Since the magnitude of the work prevented use of large numbers of animals the incidence of the hemorrhagic tendency in dogs given 400 to 600 r was reviewed *Gingival and gastrointestinal hemorrhage was common during the* second and third weeks The invariable finding at autopsy was hemorrhage into nearly all of the lymph nodes in dogs dying or killed after the 10th day, when platelets had reached extremely low levels

In the platelet transfused dogs platelet counts were not maintained at normal levels but, in general it was possible to keep the platelet counts above 100 000 per cu mm whereas the platelet counts of the control animals fell almost to zero by the ninth day The whole blood clotting time of the non transfused dog increased to 2 hours or more after the eighth day whereas the clotting time of the transfused dogs remained below 30 minutes The defect in prothrombin utilization was not corrected to as great a degree as was the clotting time Prothrombin utilization returned to normal only when the level of circulating transfused platelets remained above 180 000 per cu mm although the in vitro addition of as few as 8 000 platelets led to complete utilization in a few minutes Similarly the return of clot retraction was variable

Three dogs who received platelet transfusions were killed between the thirteenth and fifteenth days One dog died on the fourteenth day with massive bacterial invasion None of the 4 dogs showed gross or microscopic hemorrhages Two dogs showed an increased amount of hemosiderin in several lymph nodes suggesting that some bleeding had occurred possibly because in these 2 dogs the platelets had not been maintained at as high a level as in the other 2 dogs

The prevention of hemorrhage and the reversal of abnormal *in vitro* tests when the platelets are maintained at high levels by transfusion but not by platelet free plasma strongly implicates the thrombocyte deficiency as the main cause of the hemorrhagic diathesis in irradiated dogs

That factors other than the thrombocytopenia may play a part is shown by the more rapid recovery of prothrombin utilization prior to the significant elevation of the platelet count and because of alterations in the lipid antithromboplastin content of the plasma of irradiated dogs

Finally it is postulated in agreement with Furth et al [1] that there must be some change in the capillary wall before red cells can be lost from the circulation Since the platelets appear to prevent hemorrhage in the irradiated dog one might speculate that platelets normally contribute in some way to the maintenance of an intact endothelium or cement substance quite apart from their ability to plug actual holes produced by incisions

Disturbios en los Mecanismos Hemostáticos Producidos por Irradiación Total del Organismo

Desde hace muchos años la purpura generalizada y las hemorragias copiosas han sido reconocidas como un factor importante de muerte como resultado de dosis letales de irradiacion total del cuerpo

Estos disturbios hemorrágicos fueron correlacionados en el pasado por varios investigadores con la trombocitopenia que acompaña a estos estados En 1948 un nuevo concepto de hemorragias por irradiacion fue introducido por Allen y su grupo[4] que insistieron que la causa principal de la hemorragia se debía a una heparinemia y que la trombocitopenia *era un factor contribuyente* secundario Esta teoría fue puesta en duda por otros investigadores[5-9] y hoy día parece haberse satisfactoriamente eliminado como causa de hemorragia en el perro [10]

Lawrence y Valentine[11] observaron que la circulacion cruzada detenía la hemorragia en el gato irradiado trombocitopénico Brecher y Cronkite (12) demostraron que la parabiosis impide la hemorragia en el parabionto irradiado La detencion de la hemorragia mediante estos dos procedimientos podría ser explicada por deficiencia de un factor plasmático neutralizacion de un anti coagulante o incremento del numero de plaquetas Estas posibilidades fueron consideradas mediante experimentacion directa de la siguiente manera

(a) La investigacion sistemática de los factores plasmáticos conocidos demostró que no había deficiencias de protrombina globulina aceleradora [13] acelerador de la conversion de la protrombina sérica [14] factor anti hemofílico[15] y fibrinogeno [10]

(b) Anti coagulantes del tipo de la heparina fueron eliminados como factores importantes [10]

(c) El papel de las plaquetas fué investigado por estudios in vitro e in vivo

El defecto de coagulacion sanguínea producido por irradiacion en el perro correlaciona muy bien con la deficiencia de plaquetas La protrombina residual después de 60 a 37 C aumenta progresivamente a medida que el numero de plaquetas disminuye El tiempo de coagulacion aumenta después que las plaquetas caen por debajo de 50 000 por cu mm [13] La utilizacion de la protrombina es corregida in vitro por la adición de plaquetas lavadas a una concentracion final de 20 000 por cu mm

Concentrados de plaquetas aproximadamente iguales en numero a su masa normal circulante fueron transfundidos diariamente en 20 a 40 ml de plasma Cada experimento fué controlado con perros transfundidos con un volumen equivalente de plasma libre de plaquetas

En los perros transfundidos con plaquetas los recuentos de las mismas no se mantuvieron en niveles normales pero en general fué posible mantener los recuentos por arriba de 100 000 por cu mm mientras que en los perros control las plaquetas cayeron practicamente a 0 alrededor del noveno día El tiempo de coagulacion de los perros no transfundidos con plaquetas aumento hasta 2 horas o más despues del octavo día mientras que el tiempo de coagulacion de los perros que habían recibido plaquetas permaneció por debajo de 30 La utilizacion de la protrombina se hizo normal solamente cuando el nivel de plaquetas circulantes transfundidas permanecio por arriba de 150 000 per cu mm

Tres de los perros que recibieron transfusiones de plaquetas fueron muertos entre el decimotercero y décimoquinto días Un perro murio en el décimocuarto día por infeccion masiva Ninguno de los cuatro perros mostraron en la autopsia hemorragias macroscópicas ni microscópicas

La prevencion de las hemorragias cuando las plaquetas se mantienen a niveles elevados mediante transfusiones de plaquetas y la aparicion de las hemorragias cuando las transfusiones son de plasma libre de plaquetas indican ciertamente que la causa de las mismas se debería a la trombocitopenia asociada

Finalmente postulamos en concordancia con Furth y su grupo [7] que sería necesaria una alteracion de la pared capilar antes que los hematíes puedan escaparse de la circulacion Desde que las plaquetas parecen prevenir hemorragias en el perro irradiado podría espe

cularse que éstas contribuyen normalmente mediante algun mecanismo para mantener intacto al endotelio o cemento vascular aparte de su habilidad para taponar orificios producidos en los vasos por incisiones

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IV 4

Capillorrhagic and Antihemostatic Effects in Capillary Hemorrhagic Diathesis following Ionizing Radiation

ALFRED L COPLEY*

THERE have been a number of reviews[1-5] on the pathogenesis of the hemorrhagic syndrome in radiation illness I should like to present a critical appraisal of only some of the aspects which I consider significant for an understanding of radiation hemorrhage in the light of *new observations obtained in* my laboratory

Our investigations were concerned with several different approaches to hemorrhage Fundamentally these investigations have been guided by the concept of capillary hemorrhagic diathesis which I presented to you at our last Congress in Cambridge[6] The main feature of this *concept is that two major processes* each with its many facets interplay in the production of capillary hemorrhage One process concerns capillary fragility and the other hemostasis

Various tests for capillary fragility and hemostasis were applied to ascertain the effect of high ionizing irradiation in mice rats hamsters guinea pigs and rabbits Some of these tests had to be newly developed Furthermore direct *microscopic observations were made on the capillary beds of the* hamster's *pouch* and the mesentery of the rat and guinea pig

1 *Bleeding Time and Clot Resistance Studies*

The bleeding time into physiologic saline indicates the time of *bleeding from* an inflicted wound until its cessation brought about by the formation of a *wound thrombus at the site of injury* Thus the bleeding time is concerned *with the time* required to initiate hemostasis This is in contrast to clot resistance the other direct test for hemostasis Since the clot resistance test estimates the firmness of the clot and its ability to adhere to the inflicted vessel it is a measure of the maintenance of hemostasis

Mice rats and rabbits were exposed to whole body x irradiation † mainly in

* From New York Medical College New York N Y and Marine Biological Laboratory Woods Hole Mass U S A

This work was performed under Contract No AT(30 1) 780 of the U S Atomic Energy *Commission* and Contract No N7onr 40102 of the Office of Naval Research U S Department of the Navy

† The x rays administered at New York Medical College were generated in a 250 kv machine operating at 15 ma A 0 5 mm copper filter and a 1 0 mm aluminum filter were used For *all animals the dose rate 50 cm from the approximate center of* their bodies averaged 110 r per minute

The x ray machine used at the Marine Biological Laboratory was a dual tube self rectifying outfit with a simultaneous crossfiring technique The secondary voltage was

dosages of 700 and 800 r Bleeding time and clot resistance tests were performed in these animals previous to x irradiation and on various days thereafter

For the bleeding time test a mouse or rat was placed in a lucite cylinder and the tail was inserted in a tail holder which closed the cage on one end The other end near the head of the animal was closed by a perforated plunger to adjust the size of the cage to the animal This chamber was then suspended at an angle of about 40 degrees so that the tail was immersed in a physiologic saline constant temperature bath at 37 5 C The bleeding time was measured from the moment the blood was seen emerging from the wound into the saline bath until the flow of red blood stopped

For the clot resistance test a cuff pressure was applied proximal to the tail wound 5 or more minutes following the bleeding time test in order to determine whether renewal of bleeding could be induced The pressure applied always had to be appreciably below the systolic pressure of the individual subject This test, which we originally developed about 12 years ago in human subjects and mice was modified for rats and rabbits [7]

In most mice bleeding times into physiologic saline were within 2 minutes A three minute value would still be considered normal Values between 3 and 6 minutes were arbitrarily considered to be not significantly prolonged while values above 6 minutes were considered pathological

Figure 1 shows 189 bleeding times in 51 irradiated mice exposed to 700 r Prolonged bleeding times were found to occur on the 6th day following exposure From the 12th to 14th days the 6 surviving mice tested exhibited prolonged bleeding times culminating in the death of the animal

Figure 2 demonstrates repeated bleeding time tests in mice exposed to 700 r The c 4 mice which originally had normal bleeding times had abnormal values on from 6 to 12 days following irradiation One animal mouse N 30 showed a return to a normal value on the 11th day following irradiation

In contrast to bleeding time clot resistance at 60 mm of mercury cuff pressure was frequently found already decreased during the first 5 post radiation days In some animals there was a spontaneous renewal of bleeding from the tail wound previous to application of cuff pressure for the clot resistance test This also indicates that hemostasis was impaired in these animals

The frequency occurrence of 265 bleeding times in 119 normal rats is shown in figure 3 Here about 94 per cent of the tests have bleeding times within 90 seconds and 99 per cent show bleeding times within two minutes This was further substantiated in results of 498 bleeding times on 241 rats not presented in figure 3 Most bleeding time tests which we performed in healthy human subject using the principle of bleeding into saline [8] were also within 2 minutes

Figure 4 shows 307 bleeding times in 78 rats exposed to 700 r Prolonged bleeding times were never found before the 4th post radiation day Repeated

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180 kv and the tube current on each tube was 25 ma The heavy glass of the tube walls and 5 mm bakelite of the tube shields gave the filtering value of 0.2 mm of copper shield The intensity was 111 r per minute at a distance of 6 7 cm

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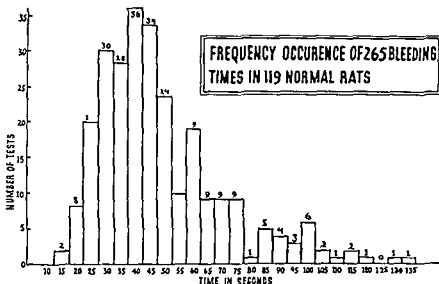


Fig 3

bleeding time tests in 2 infant rats exposed to 700 r are charted in figure 5 Attention should be drawn to rat VII-43 which had normal bleeding times on the third fifth and sixth day while on the seventh day a bleeding time of 39 minutes was measured At the end of this test the animal was found to be dead It bled to death from a single wound in the tail The other animal rat IX 132 shows a normal bleeding time on the sixth day and two markedly prolonged bleeding times on the 7th and 8th days

One animal x irradiated with 800 r had on the 2nd post radiation day a positive clot resistance and a normal bleeding time of 76 seconds On the 3rd post radiation day the bleeding time was 31 5 minutes

A hemorrhagometer was developed for rabbits and is diagrammed in figure 6 A uniform prick wound was inflicted in the rabbit s forepaw and a cuff pressure was applied proximally The frequency occurrence of 83 bleeding times in 43 normal rabbits is shown in figure 7 Seventy eight per cent of these bleeding times are within 2 minutes Results of 116 bleeding times in 64 rabbits not charted in figure 7 also showed a similar distribution In comparison with the bleeding times in normal rats and mice many normal rabbits had wider variations between repeated bleeding time determinations

Out of 202 bleeding times in 41 rabbits x irradiated with 800 r 17 were prolonged above 6 minutes Two of these rabbits exhibited prolonged bleeding times already on the 1st post radiation day one on the third while the remaining bleeding times were prolonged on the fifth to the ninth post radiation days

A summary of prolonged bleeding times of 6 minutes or more and of decreased clot resistance in x irradiated mice rats and rabbits is shown in table 1 Clot resistance was found decreased frequently in all species within the first four days after irradiation With increasing x ray dosage up to 2100 r decreased

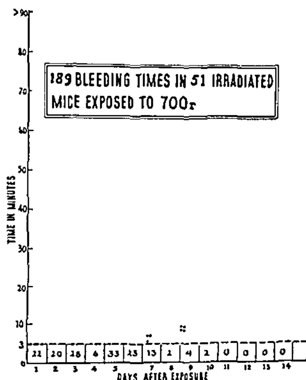

189 BLEEDING TIMES IN 51 IRRADIATED MICE EXPOSED TO 700r
TIME IN MINUTES
DAYS AFTER EXPOSURE

Fig 1

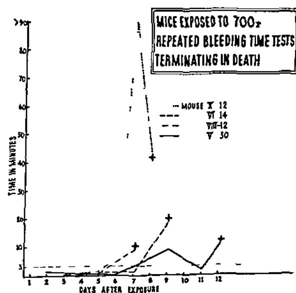

MICE EXPOSED TO 700r
REPEATED BLEEDING TIME TESTS
TERMINATING IN DEATH
MOUSE X 12
VI 14
VIII-12
V 30
TIME IN MINUTES
DAYS AFTER EXPOSURE

Fig 2

HEMORRHAGOMETER FOR RABBITS

Fig 6

FREQUENCY OCCURRENCE OF 83 BLEEDING TIMES IN 43 NORMAL RABBITS

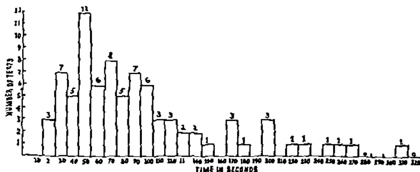


Fig 7

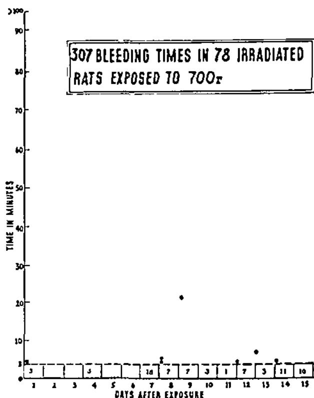
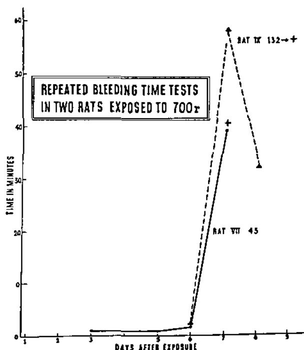

307 BLEEDING TIMES IN 78 IRRADIATED RATS EXPOSED TO 700r
TIME IN MINUTES
DAYS AFTER EXPOSURE

Fig 4


REPEATED BLEEDING TIME TESTS IN TWO RATS EXPOSED TO 700r
TIME IN MINUTES
DAYS AFTER EXPOSURE

HEMORRHAGOMETER FOR RABBITS

Fig 6

FREQUENCY OCCURRENCE OF 83 BLEEDING TIMES IN 43 NORMAL RABBITS

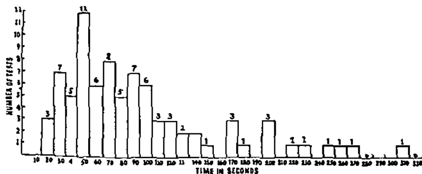


Fig 7

TABLE 1—*Markedly Prolonged Bleeding Time and Decreased Clot Resistance in Mice Rats and Rabbits following Whole Body X irradiation*

Findings	Species	Dose	Days after x irradiation															
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Prolonged bleeding time of 6 min or more	Mice	700						+	+	+	+	+		+	+	+		
	Rats	700				+	+	+	+	+	+	+	+	+	+	+	+	
	Rabbits	800	+		+		+	+	+	+	+	+						
Decreased clot resistance	Mice	700	+	+	+	+	+	+	+	+	+	+	+	+		+		
	Rats	700	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+
	Rabbits	800	+	+	+	+	+	+	+	+								

clot resistance was more frequently found in the three species tested Our findings of the early occurrence of decreased clot resistance frequently observed on the first and second days after exposure indicate impaired hemostasis, often long before other tests for hemostasis might show abnormal results This hemorheological test may prove to be useful in human beings in the early *diagnosis* of radiation injury

2 *Blood Coagulability*

Because blood coagulation has been mistakenly assigned the central role in capillary hemostasis the nature of radiation produced capillary hemorrhagic diathesis is universally misunderstood Any hemorrhagic agent including high ionizing irradiation does not necessarily have an anticoagulant effect [6] In the light of this it is not paradoxic that we found massive coagulation thrombi in the heart and in the large blood vessels immediately after the death of animals with x ray produced hemorrhagic diathesis

Since it was reported in the recent literature that a heparin like substance was found in the circulation following x irradiation [10] we endeavored to test the *coagulability of blood* in x irradiated rabbits by means of the blood saline coagulation test [11, 12] Figure 8 shows a frequency distribution of 59 blood saline coagulation time tests in 48 normal rabbits The blood was diluted serially up to 64 times with physiologic saline One animal showed a coagulation time of 13 minutes in the 12 5 per cent blood concentration a trend toward hyper coagulability

Figure 9 exhibits the frequency distribution of 55 blood saline coagulation tests in 32 x irradiated rabbits one to fifteen days following exposure to 700 r or 800 r From this diagram it becomes apparent that in no single test was the *coagulation time* in the 100 per cent blood concentration significantly prolonged the highest values being less than 11 minutes Hypocoagulability could be detected in one test at 50 per cent and 25 per cent blood concentrations Hyper coagulability on the other hand is evident in three tests at 12 5 per cent blood concentration as well as in the 6 3 per cent concentration in three tests and at

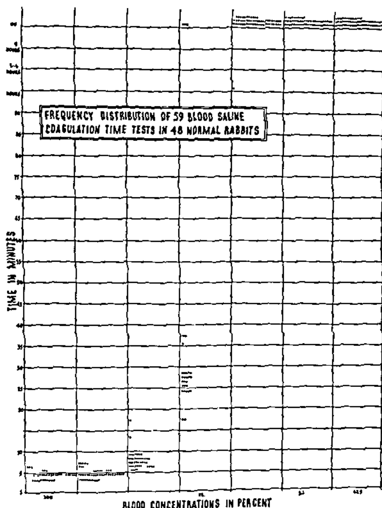


Fig 8

3 1 per cent in two tests In these instances no values were obtained at the 1 6 per cent blood concentration

Figure 10 illustrates the few abnormal blood coagulability values which were obtained in four animals A fifth rabbit exposed to 700 r but not on this chart showed hypercoagulability One rabbit XII-4 exposed to 800 r exhibited a typical normal coagulability on the first day following irradiation while the same animal showed definite hypocoagulability on the 6th day Of the other three animals which were all exposed to 700 r, only rabbit V-4 showed a trend toward hypocoagulability on the seventh day as shown in the value of 30 minutes in the 12 5 per cent blood concentration The two other animals exhibited hypercoagulability one animal rabbit V 14 on the first day the other animal rabbit IV-S 2 on the 8th day

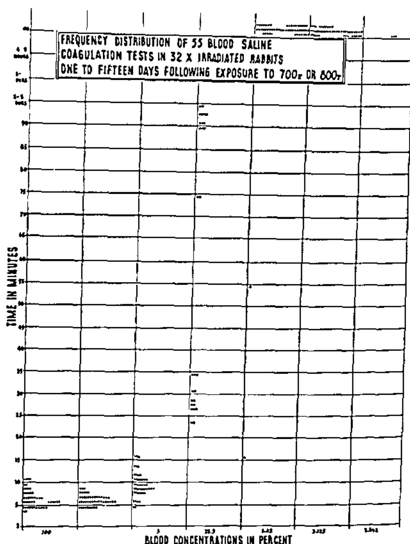


Fig 9

In preliminary studies calcium determinations were made in rabbit serum Previous to x irradiation values for calcium ranged from 9 1 to 11 7 mg per cent Increases in total serum calcium were found up to 50 per cent as early as the 2nd post radiation day In one case an increase of 62 per cent was observed on the seventh post radiation day Occasionally a return to the control value was observed on the sixth post radiation day However we did not always find increases in total serum calcium values The question arises whether there is usually an increase in ionized calcium following x irradiation even though there may be no increase in total calcium

Our interest in calcium determinations in x irradiated animals arose from findings on the effect of calcium salts on blood coagulability Minute variations in amounts of calcium at physiologic levels were found to produce either hyper

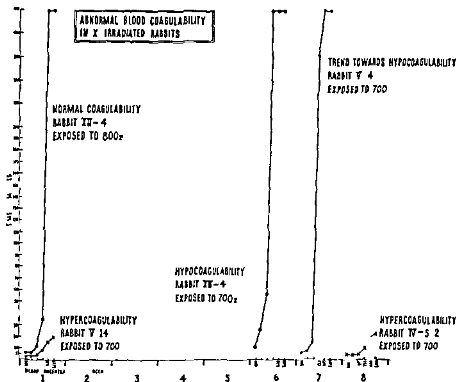


Fig 10

coagulability or hypocoagulability [13 14 15] It is very possible that this geloplastic and antigeloplastic effect[15] of calcium variations may explain the contradictory findings

3 Firmness of Whole Blood Coagula and Platelet Agglutinates

The effect of whole body x irradiation on the relative firmness of blood coagula of rabbits exposed to 800 r was studied Blood samples free of tissue juice were obtained from the heart and placed in specially designed incubation tubes At intervals varying from 1 to 80 minutes samples were removed from incubation at 37 5 C A *special glass viscometer with an orifice of 1 3 mm internal diameter* was used Air pressure was applied and the firmness was recorded as mm Hg pressure at the time the clot was forced through the viscometer undergoing de formation from a maximum of 8 mm internal diameter to one of 1 3 mm

It was found from 161 control tests of blood samples secured from 15 rabbits that the firmness of blood coagula increases with time Only a few very low *pressure values were found three minutes after gelation and only 0 pressure* values could be obtained earlier than this It was more usual however to find 0 pressure values up to six minutes of incubation and a gradual increase up to 11 minutes at which time there was a sharp rise to a high firmness of the coagula

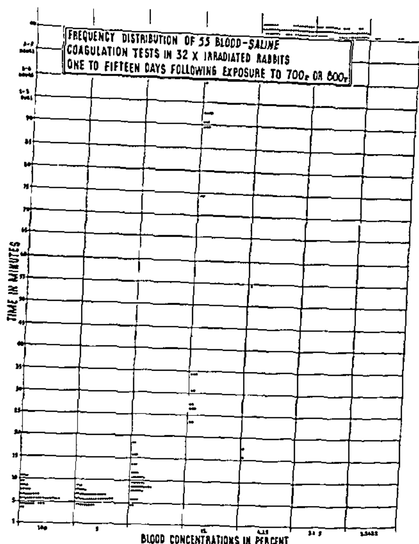


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TABLE 2—*Comparison of Findings of Platelet Agglutination Thrombi and Emboli in the Cutaneous Capillary Vessels of Cheek Pouches of Hamsters Irradiated with 725 r and of Platelet Counts Taken from Heart Blood*

Postradiation day	Number of hamsters	In vivo				In vitro	
		Occurrence of single platelets	Platelet agglutinates and signs of stasis within 3 hours after pouch preparation: Thrombi	Platelet agglutinates and signs of stasis within 3 hours after pouch preparation: Emboli	Embolizing red cell masses	Average platelet counts × 10³	Platelet agglutinates
0	10	2+ or 4+	0	0	0	654	0
1	4	+ or 2+	3+	3+	0	350	P
2	5	+ also seen to adhere to endothelium	3+	3+		235	P
3	3	+	0 + or 3+	0 or +	0	154	0
4	1	Capillary bed obscured by bleeding				68	0
5	6	+ or 3+	Hyaline	Hyaline		151	0
6	5	+ or 0	0	0	0	72	0
7	3	+	1 mixed red and white thrombus	0	P	132	0
8	3	+ *or* 0	0	0	P	58	0
9	2	0	I (insufficient to plug wounds or vessels)	P	P	28	0
10	1	+	0	0	0	72	0
11	2	+	0 or 3+	0 or 3+	P	92	0
12	2	+ or 2+	0 or hyaline	0 or hyaline	1		
13	1	+	0	0	0		
16 23	3	+ or 4+	0 or P	0	0		

0 = none + = occasional 2+ = moderate 3+ = many 4+ = numerous P = present

Phase contrast microscopy was not employed in these observations because the thickness of the tissue did not permit its use The double diaphragm method of oblique illumination (DD) proves to be a better tool for direct observation of blood cellular elements in the circulation and the endothelium of capillary vessels than the usual bright field illumination[7]

In table 2 findings are compiled on a comparative study of observations in 40 hamsters x irradiated with 725 r and of platelet counts[18] taken from the hearts of the same animals immediately after the in vivo observation periods of 2 hours were completed

During the first and second post radiation days platelet agglutinates could also be observed in the counting chamber They were in small groups of 2 to 5 platelets which were included in the count No larger ones were seen although numerous large agglutinates containing hundreds of platelets were observed in the circulation

The single platelets were best seen in the smallest capillary vessels of 5 μ to 10 μ diameter They could also occasionally be seen in the plasma zones at junctions where there was slowing down of the red cell stream and eddy formation

One hundred and seventy seven comparative tests on 14 x irradiated rabbits were made through the 9th post radiation day

These tests indicated that there is a marked increase both in the firmness and in the rate of development of firmness of blood coagula from these x irradiated rabbits After approximately 8 or 9 minutes firmness of coagula increases at least three fold within a 30 second interval It was also noted that clots from these x irradiated rabbits are much more elastic than those from their controls Syneresis was found to occur to a lesser extent in x irradiated than in normal rabbits concurring with the results of earlier investigations[2, 4]

The great increase in clot firmness following x irradiation is not well understood Of interest is the finding by Cronkite[16] of a frequent increase in fibrinogen following x irradiation Future experimentation is necessary to correlate if possible, this increase with the increase in clot firmness There might also be an effect of substances which are contained in tissue juice and would enter the circulation in radiation injury In preliminary observations in hemophilic blood Ehrlich and Copley[17] found that tissue juice increased clot firmness

Of significance is the finding that despite low platelet counts and decreased retraction the firmness of coagula from x irradiated animals is markedly increased This emphasizes an earlier conclusion that contrary to the general opinion a non retracted or slightly retracted coagulum of a thrombocytopenic blood sample can show a firmness higher than normal

The differentiation between a clot composed of platelet agglutinates and a clot produced by the coagulation of whole blood is most important in a consideration of capillary hemostasis Arrest of hemorrhage in blood capillaries is mainly a function of the agglutination of platelets and not of fibrin formation while hemostasis in the large blood vessels it brought about mainly by plasma coagulation Because of this differentiation we studied the firmness of platelet agglutinates in comparison with the firmness of blood coagula of the same blood withdrawal care being taken not to admix tissue juice Platelet counts were compared with these firmness data These counts were made according to the method of Brecher and Cronkite[18] but using the Wild phase contrast microscope

Platelet agglutinates were produced by the addition of large amounts of heparin to blood samples followed by centrifugation These agglutinates from normal rabbit blood exhibited high firmness when deformed through micro viscometers Following exposure of rabbits to 800 r the platelet agglutinates which were recoverable tended to become smaller and less firm The agglutin ability of the platelets was found to decrease although sufficient numbers of platelets were present No platelet agglutinates could be recovered on the seventh and ninth post radiation days by the heparin method Yet this method was successful in recovering platelet agglutinates from animals with significantly lower platelet counts which were tested during the first 4 post radiation days[19]

4 *The Genesis of Thrombocytopenia*

Direct microscopic observations of the capillary bed of the hamster's cheek pouch were made according to the procedure described earlier at this Congress[7]

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12	2	+ or 2+	0 or hyaline	0 or hyaline	I		
13	1	+	0	0	0		
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Phase contrast microscopy was not employed in these observations because the thickness of the tissue did not permit its use The double diaphragm method of oblique illumination (DD) proves to be a better tool for direct observation of blood cellular elements in the circulation and the endothelium of capillary vessels than the usual bright field illumination [7]

In table 2 findings are compiled on a comparative study of observations in 40 hamsters x irradiated with 725 r and of platelet counts[13] taken from the hearts of the same animals immediately after the in vivo observation periods of 2 hours were completed

During the first and second post radiation days platelet agglutinates could also be observed in the counting chamber They were in small groups of 2 to 5 platelets which were included in the count No larger ones were seen although numerous large agglutinates containing hundreds of platelets were observed in the circulation

The single platelets were best seen in the smallest capillary vessels of 5 μ to 10 μ diameter They could also occasionally be seen in the plasma zones at junctions where there was slowing down of the red cell stream and eddy formation

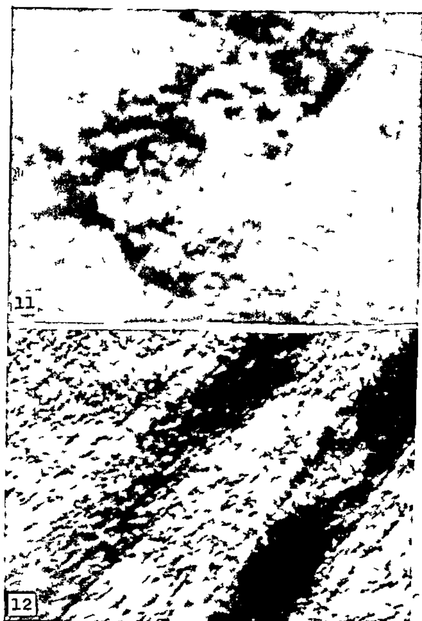


FIG 11—Platelet agglutination thrombus lodged at branch of vein in cheek pouch of hamster exposed to 725 r six hours after irradiation Note large platelet agglutinate entirely obstructing bifurcation of vessel DD microscope 450 ×

FIG 12—Platelet agglutination embolus (E) in artery and thrombus (T) in vein of hamster s pouch on third post radiation day Many nonagglutinated platelets not shown in the photograph were seen in smaller vessels No new deposition of platelets on thrombus was seen during observation of approximately 15 minutes DD microscope 160 ×

The thrombi were seen with only one exception in the venous vessels of the capillary bed in the irradiated animals The most striking findings are the occurrences of emboli and thrombi as early as 5 hours after irradiation (fig 11)

In 10 control hamsters the injury caused by the removal of connective tissue during which operation many small vessels were broken caused a transient thrombo embolization at the sites of injury The emboli in the circulation disappeared within 15 minutes Stasis resulting from lodged emboli during this period disappeared in approximately the same time Injuries which occurred later, whether accidentally or experimentally produced showed the same general picture in normal animals

In irradiated hamsters during the first few post radiation days however platelet agglutination thrombi and emboli were seen during the whole time of observation (fig 12) Thrombo-embolization occurred at sites in the venous circulation in which no break was manifest However very long platelet thrombi which were sites of embolus formation were seen on the venous vessel walls extending up to several hundred microns in length Platelet agglutination emboli were seen to peel off and be carried away in the stream Large emboli were seen in the arterial circulation which lodged in the smaller vessels Possibly this is why in blood withdrawals from the hearts of irradiated hamsters during the first few post radiation days large platelet agglutinates were not usually seen in the counting chamber However very large platelet agglutinates have often been found in the hearts of irradiated rabbits

The occurrence of hyaline thrombi and emboli in which the outlines of the individual platelets no longer are visible and the finding that no platelets are deposited on thrombi from the fifth to the twelfth day suggest that these bodies might be old formations of platelet agglutinates which have persisted from the first post radiation days

It appears especially significant that in the period immediately following the trauma produced by the preparation of the pouch during the fifth to eighth post radiation days no thrombi or emboli were seen in the circulation with the exception of rare hyalinized bodies and in one instance on the 7th post radiation day a mixed red and white thrombus The relatively rare platelets which were observed during this period did not agglutinate Agglutination of platelets and thrombi was detected again from the 16th to the 23rd post radiation days during which period the number of visible single platelets in the circulation appeared to be increased

Of interest are observations of embolizing red cell masses following irradiation occurring most markedly from the 7th to 12th post radiation days During this period many animals died Therefore further observations on platelets during later post radiation days could not be made At the dosage of 725 r employed in this study there was also a high mortality during the early post radiation days

No comparative studies were made of white cell counts and the appearance of white cells in the circulation However we observed increased leukocytic pavements during the first two post radiation days followed by a marked decrease in white cells in the circulation Leukocytes reappeared around the 12th post

radiation day and also were seen adhering to the venous walls It is not certain whether this occurrence of pavements indicates an adhesive property of the endothelium or of the leukocytes

Our observations on the development of platelet agglutination thrombi may explain the genesis of thrombocytopenia in severe radiation injury The common contention has been that platelet production is impaired, because of the damage or reduction in number of the megakaryocytes whenever other elements in the bone marrow showed injury The new explanation which we propose is that platelets agglutinate mainly in the venous side of the capillary bed and hyalinize within short periods of time Since a large part of the platelet population is involved in this process of plugging large vessels in the capillary circulation, and of subsequent disintegration due to hyalinization thrombocytopenia results The damaged and reduced megakaryocytes then are no longer capable of replenishing the supply of circulating platelets Moreover any such supply and release into the circulation may further be diminished in the early post radiation period by continuous platelet agglutination in the capillary beds It is quite possible that severe radiation injury produces circulating platelet agglutinant substances Thus, detailed studies following irradiation, both on observations of platelets in capillary beds, as well as in vitro studies of platelet agglutinant activity of serum or plasma from irradiated animals may explain the pathogenesis of thrombocytopenia in radiation injury

Findings of hypoagglutinability of platelets during late post radiation days[19] raises the question whether or not after initial hyperagglutinability of platelets due to possible increase of platelet agglutinant substances in the circulating blood a condition of platelet hypoagglutinability was produced due to a possible increase of platelet antiagglutinant substances [20] Platelet agglutinant substances were found[21] in globulin fractions mainly in gamma globulin while the presence of platelet antiagglutinant substances in the circulating plasma was merely hypothesized [20]

Since platelet hypoagglutinability appears to occur in later stages of severe radiation injury then the generally observed onset of severe hemorrhages after about one or more weeks following exposure could be explained not *merely on the basis of thrombocytopenia alone but* in addition on the hypo agglutinability of the reduced platelet population

5 *Capillorrhagic Studies*

Any agent which brings about the rupture of a capillary blood vessel was termed capillorrhagic Capillary hemorrhage was produced by means of physical and chemical agents Negative pressures at various increments were applied to the capillary bed of the guinea pig s and rat s mesentery and of the hamster s pouch with specially constructed lucite pressure cups or blunted hypodermic needles Various chemical hemorrhagic agents were injected by different routes or applied topically

From our comparative observations of the mesentery in rats and guinea pigs we found the guinea pig preparation more suitable A block made of

lucite was found very useful to facilitate viewing at higher magnifications It was possible to observe the mesentery of the living animal at 1000× with clarity

In control guinea pigs the application of negative pressures of −300 mm Hg or more produced stasis Flow resumed first in the arterioles and was followed by resumed flow in the venules from one half to two minutes following release of suction When pressure of −500 mm Hg was applied for several seconds a definite break in the vessel wall was produced resulting in extravasation Topical application of brilliant cresyl blue which is a differential stain for platelets revealed a platelet lining in the vessel just behind the break which became thicker as it neared the break A very narrow path through these plate let masses allowed some blood flow (Systemic injection of brilliant cresyl blue was also found to stain platelets) Larger and more pronounced breaks were found in venules, which were plugged by agglutinated platelet mas es through which small openings permitted further extravasation of blood

In irradiated animals, extravasation was produced a short distance from the area where the negative pressure was applied The pressure area also contained hemorrhagic manifestations The negative pressure necessary to produce these hemorrhages in irradiated animals was considerably below the negative pres sures required for the control animals Pressures of about −200 mm Hg oc casionally produced breaks in the walls of arterioles

We found the preponderance of hemorrhage in the small venules which drain the capillary bed in the guinea pig s mesentery and in the hamster s pouch Petechiae and ecchymotic plaques were found to occur less often around small arterioles and true capillaries Since collagen bundles are found in greater abundance adjacent to the small venules than to the true capillaries the ques tion arises whether a weakening of collagen may be considered as a causative

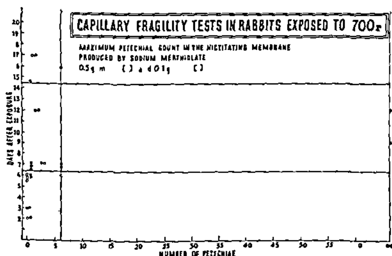


FIG 1

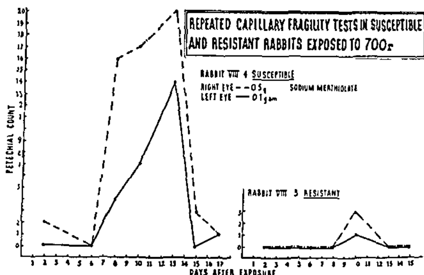


Fig 14

factor in the decreased strength of these venules Dilation of these small collecting venules is often observed along with sluggishness of blood flow This may be an additional factor in lowering further the decreased strength of their walls In studies of animals in which a thoraco lumbar sympathectomy was performed[7] decreased clot resistance was found

Capillary fragility tests[22, 23, 24] were made in the nictitating membrane of the eye of rabbits exposed to 700 r (fig 13) During an observation period of three hours following injection of 0 1 and 0 5 gamma sodium merthiolate into the nictitating membrane petechiae were counted and the occurrence of ecchymosis was noted On this chart the maximum petechial counts are listed Increased petechial counts were observed only from the 7th to 14th day after exposure A petechial count of less than 6 was considered to be normal

Figure 14 exhibits findings of repeated capillary fragility tests in a susceptible and a resistant animal In the susceptible animal increased petechial counts were found from the 7th to 13th day after exposure

Of interest are observations of the capillorrhagic effect of fibrinolysin which was not known hitherto [2] Following injection of fibrinolysin into the nictitating membrane of rabbits large numbers of petechiae suddenly appeared From these results Copley implied that if the fibrinolytic agent of the fibrinolysin preparation ruptures capillary vessels then possibly there is fibrin in the inter endothelial cement or in a coating on the entire inner endothelial wall These observations gain added importance because of findings by Cronkite that serum fibrinolysin may be activated in acute radiation illness[3] Our new concept on the physiologic role of fibrin [5] may relate capillary hemorrhage to high fibrinolytic activity

6 *Comparative Hemorrhagic and Purpuric Tests*

Comparative studies on defective hemostasis and purpura were made in X ir radiated rabbits[25] The degree of capillary hemorrhagic diathesis was estimated

by the occurrence of ecchymotic plaques produced by sodium merthiolate in the nictitating membrane in which petechial counts were also made to detect increase of capillary fragility Impaired hemostasis was estimated by the oc *currence of prolonged bleeding times and decreased clot resistance* These purpuric and hemorrhagic tests were done within 6 to 8 hours

In order to perform the capillary fragility and ecchymosis tests on as many animals with prolonged bleeding times as possible the x ray dose was increased to insure that the animals which lived long enough would have prolonged bleeding times Out of 23 rabbits irradiated with 1500 r 13 surviving animals were tested Seven had bleeding times of 10 minutes or more Three of these rabbits exhibited both increased capillary fragility and ecchymosis on the sixth or seventh post radiation day when their bleeding times were prolonged Two animals had normal or insignificantly increased petechial counts on the day that they had markedly prolonged bleeding times over 1 5 hours on the sixth post radiation day in one animal and 1 3 hours on the ninth post radiation day in the other Two animals had increased petechial counts but no ecchymosis when their bleeding times were more than 12 minutes One of these rabbits was retested 2 days later when there was a bleeding time of over 1 5 hours and upon injection of sodium merthiolate ecchymosis During the first two hours of the nictitating membrane tests no increased petechial count was noted and the rabbit died at the end of this period

Thus our contention[6] that the simultaneous occurrence of impaired hemo stasis and increased capillary fragility would result in capillary hemorrhagic diathesis was borne out by these and other studies in rabbits In most instances ecchymosis was produced when capillary fragility was increased and bleeding times were markedly prolonged or the clot re istance was decreased However there were cases in which the bleeding time was increased the petechial count was considerably higher than normal but no ecchymosis was produced In one case on a subsequent day the bleeding time was more than 1 5 hours and ecchymosis became manifest This latter case demonstrates that a condition existed on *the previous day* which we should like to designate as latent capillary hemorrhagic diathesis

Since the early observations made by London [6] in 1903 it has been known that irradiated animals may die without or with only very slight injuries to the skin. Our results are of special significance because they concur with the hematologic findings in severe radiation injury in man resulting from exposure of the whole body to the initial nuclear radiations of an atomic bomb recently summarized and analyzed by Dunham Cronkite LeRoy and Warren [27] These authors *found that hemorrhagic manifestations in various organs in patients* whose exposure to radiation was not sufficient to cause severe radiation injury were not necessarily always accompanied by cutaneous purpura However those patients with cutaneous purpura and severe radiation injury displayed other *hemorrhagic manifestations Since these authors considered radiation* injury in man to be equivalent to the type of injury that occurs in experimental mammals exposed to a comparable dose of x rays it seems to us that our com parative findings on ecchymosis increased capillary fragility and impaired

hemostasis mentioned above are of special importance in relation to the observations that cutaneous purpuric manifestations in atomic bomb injuries may not accompany bleeding in other organs. Therefore, what we designate as latent capillary hemorrhagic diathesis, appears to refer to cutaneous capillary hemorrhagic diathesis and not necessarily to non-cutaneous capillary hemorrhagic diathesis.

There might be factors in the cutaneous capillary beds residing mainly in the pericapillary sheath(s) and connective tissue matrix which would increase the strength of the capillary wall[6] and also aid in hemostasis. Such factors may not be present to the same degree in visceral capillary beds. For these reasons, it becomes important to compare studies in the cutaneous and visceral as well as other non-cutaneous capillary beds—preferably in the same species—in cases of acute radiation injury.

In the light of our findings, clot resistance studies in patients with acute radiation injury appear warranted. Other standardized methods of petechial counts for studying capillary fragility and of ecchymosis for estimating capillary hemorrhagic diathesis[8] are available for comparative studies in patients with radiation injury. These investigations should include studies on platelet agglutinability and on hemorheological[29] properties of platelet agglutinates and blood coagula such as adhesiveness to foreign surfaces and firmness.

Our varied studies have been presented in an attempt to clarify and interrelate the diverse capillorrhagic and antihemostatic effects from acute radiation injury in an endeavour to advance our knowledge on the pathogenesis of radiation hemorrhage.

Efectos Anti-Hemostaticos y Capilorragicos en la Diatesis Hemorragica Capilar por Radiaciones Ionizantes

Ha habido una serie de puestas al día[1–6] sobre el tema de la patogénesis del síndrome hemorrágico en enfermedades por irradiación. Nuestras investigaciones se refieren al estudio de las hemorragias en esta situación especial. Fundamentalmente estas investigaciones se han basado en el concepto de la diátesis hemorrágica capilar presentadas en el ultimo Congreso de Cambridge.[8] El punto principal de este concepto es que hay dos procesos primordiales, cada uno con varias facetas, que se relacionan con la producción de hemorragias capilares. El primero se refiere a la fragilidad capilar y el segundo a la hemostasia.

Estos factores fueron estudiados desde el punto de vista de su alteración por los efectos de altas irradiaciones ionizantes mediante un numero de tests en lauchas, ratas, hamsters, conejillos de India y conejos. Tambien se hicieron observaciones microscopicas en los lechos capilares del "hamster's pouch" y del mesenterio de la rata y del conejillo de la India.

Tiempo de sangría y resistencia del coágulo

Varios experimentos se hicieron para investigar el tiempo de sangría y la resistencia del coágulo en ratas y conejos irradiados. El primero se hallo aumentado y la resistencia del coágulo disminuída, esto ultimo frecuentemente en el primer y segundo días, indicando una hemostasia alterada.

Este test sería muy util en seres humanos para el diagnóstico precoz de enfermedad por irradiacion.

Coagulación Sanguínea

En cuanto a la coagulacion sanguínea se observo que, contrariamente a lo aceptado por la mayoría de los autores, el tiempo de coagulacion no se halla aumentado en las condiciones en que se hicieron los experimentos.

El nivel del calcio en sangre parece tener un papel importante habiendose econtrado en algunos casos un aumento hasta del 62%

Firmeza del coágulo

Hay aumento de la firmeza y de la velocidad de obtención del coágulo en conejos irradiados Esto no ha sido bien comprendido pudiendo explicarse por el hallazgo de que el fibrinógeno aumenta en los animales irradiados o por el pasaje de alguna substancia tisular a la circulación como resultado de la irradiacion

De interés es el hecho de que a pesar de haber trombocitopenia y disminución de la retracción del coágulo la firmeza de éste en animales irradiados es mayor

Se consideró también la diferencia entre el coágulo capilar y el de los vasos mayores En el primer caso se debe a aglutinados de plaquetas y no a la fibrina mientras que en los vasos mayores se forma por coagulacion de la sangre

Genesis de la trombocitopenia

Nuestras observaciones sobre la formacion de los trombos de plaquetas aglutinadas puede muy bien explicar la génesis de la trombocitopenia en las irradiaciones severas El concepto comunmente aceptado hasta la fecha explica la trombocitopenia por medio del dano o reduccion de los megacariocitos en la médula osea La nueva teoría que proponemos es la de que las plaquetas se aglutinan principalmente en la parte venosa del lecho capilar y se hialinizan rápidamente Como la mayor parte de las plaquetas se ven envueltas en este proceso de taponamiento en la circulación capilar con su subsiguiente desintegracion debido a hialinización se obtiene como resultado la trombocitopenia Como segundo paso los megacariocitos danados y reducidos en numero se hallan incapaces de reemplazar la pérdida de plaquetas Es muy posible que la irradiacion severa produzca substancias plaqueto aglutinadoras en la circulacion

En los días que siguen a las primeras reacciones se ha encontrado una hipoaglutinabilidad de las plaquetas lo cual plantea el interrogante sobre si después de un período inicial de hiper aglutinabilidad de las plaquetas debido posiblemente al aumento de substancias plaqueto aglutinadoras en la sangre circulante seguiría un periodo de hipo aglutinabilidad debido a substancias anti plaqueto aglutinadoras

Desde que la hipo aglutinabilidad plaquetaria parece ocurrir en los períodos tardios de la irradiacion severa la causa de las hemorragias las cuales aparecen entre una y dos semanas despues de la exposicion podría explicarse no solamente sobre la base de la trombocitopenia pero también por la hipo aglutinabilidad de las plaquetas reducidas

Estudios capilorrágicos

Los estudios de la fragilidad capilar fueron hechos en la membrana nictitante de los ojos de conejos irradiados y luego inyectados con 0 1 y 0 5 microgramos de mertiolato de sodio y contando luego el numero de petequias y la aparicion de equimosis Recuentos de petequias por debajo de 6 se consideraron normales El numero de petequias que aparecio despues de ejecutados los experimentos fue elevado especialmente entre el 4 y 14 días

La inyeccion de fibrinolisina en dicha membrana trajo la rápida aparicion de petequias Creemos que las hemorragias capilares pueden deberse a una accion fibrinolítica aumentada

Estudio comparado de los tests purpuricos y hemorrágicos

El grado de diátesis hemorrágica capilar se estimó mediante la aparicion de manchas equimoticas producidas por mertiolato de sodio en la membrana nictitante en la cual también se hicieron recuentos de petequias para medir el aumento de la fragilidad capilar La alteracion de la hemostasia se estimó mediante la presencia de tiempos de coagulacion aumentados y resistencia disminuída del coágulo

Como resultado de todos estos experimentos nace nuestra *teoría de que la alteracion de la hemostasia y la fragilidad capilar aumentada serían la causa de la diátesis hemorrágica*

hemostasis, mentioned above, are of special importance in relation to the observations that cutaneous purpuric manifestations in atomic bomb injuries may not accompany bleeding in other organs Therefore what we designate as latent capillary hemorrhagic diathesis, appears to refer to cutaneous capillary hemorrhagic diathesis and not necessarily to non cutaneous capillary hemorrhagic diathesis

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capilar En la mayoría de los casos la equimosis se produjo cuando la fragilidad capilar estaba aumentada y los tiempos de sangría marcadamente prolongados o la resistencia del coágulo disminuída Sin embargo hubo casos en los cuales el tiempo de sangría estaba prolongado el recuento de petequias considerablemente por encima de lo normal pero sin producción de equimosis En un caso, en un día subsiguiente el tiempo de sangría fue *mayor de una hora y media* y las equimosis se hicieron manifiestas Este último caso demuestra que existió una condición en el día previo que nos gustaría llamar diátesis hemorrágica capilar latente

Desde las tempranas observaciones hechas por London[16] en 1903 se sabe que animales irradiados pueden morir con ausencia o muy leves lesiones de la piel Nuestros resultados son de una significación especial porque concuerdan con los hallazgos hematológicos en los casos de lesiones por irradiación severa en el hombre resultantes de la exposición a cuerpo entero a las radiaciones nucleares iniciales de la bomba atómica recientemente resumidas y analizadas por Dunham Cronkite Le Roy y Warren[27] Estos autores encontraron que las lesiones hemorrágicas en varios de los órganos de estos pacientes cuya exposición a la irradiación no fué suficiente para causar lesiones severas no fueron necesariamente acompañadas siempre con purpura cutánea Como en la opinión de estos autores la lesión *por irradiación en el hombre se considera equivalente al tipo de lesión que ocurre en mamíferos* cuando experimentalmente se los expone a una dosis comparable de rayos X nos parece que nuestros hallazgos comparativos concernientes a equimosis fragilidad capilar aumentada y alteraciones de la hemostasia arriba mencionadas son de especial importancia con relación a las observaciones de que las manifestaciones purpuricas cutáneas en las lesiones por la bomba atómica pueden no acompañar a las manifestaciones hemorrágicas de otros órganos Por lo tanto lo que nosotros designamos como diátesis hemorrágica capilar latente se referirá a la diátesis hemorrágica capilar cutánea pero no necesariamente a la diátesis hemorrágica capilar extra cutánea

Existirían factores en los lechos capilares cutáneos ubicados principalmente en la capa peri capilar y en el tejido conectivo que aumentarían la resistencia de la pared capilar[6] y que también ayudarían en la hemostasia Estos factores podrían estar ausentes o disminuídos en los lechos capilares viscerales Por estas razones se hace importante el estudio comparativo de los lechos capilares cutáneos y viscerales en los casos de lesiones agudas por irradiación

Segun nuestros estudios creemos de suma importancia en este tipo de paciente la investigación de la resistencia del coágulo Otros métodos standardizados para recuentos de petequias con el objeto de estudiar la fragilidad capilar y de equimosis para estimar la diátesis capilar hemorrágica[28] han sido puestos al alcance de los investigadores para estudios comparativos en sujetos con lesiones por irradiación Estas investigaciones deberían incluir estudios sobre la aglutinabilidad de las plaquetas y de las propiedades de estos aglutinados plaquetarios y coágulos sanguíneos tales como su adhesividad a superficies extrañas y firmeza

Estos varios estudios han sido presentados en un intento de clarificar e interrelacionar los diversos efectos anti hemóstáticos y capilorrágicos producidos por lesiones agudas por irradiación con la intención de avanzar nuestros conocimientos en la patogénesis de dicha enfermedad

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PART V

Polycythemia

Policitemia

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[28] COPLEY A L AND KOZAM G Capillary fragility and the ecchymosis test in man J Appl Physiol 4 311 1951
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La Policitemia de la Altura

ALBERTO HURTADO*

VIAULT,[1] en 1892 demostró la existencia de policitemia en los habitantes de una población andina en el Perú. Desde esta observación original, numerosas investigaciones llevadas a cabo en las grandes alturas y en cámaras de baja presión han confirmado que una disminución en la tensión parcial del oxígeno en el aire inspirado origina, entre otros procesos, un aumento en los hematíes y hemoglobina circulantes. La creciente importancia de los problemas médicos de la aviación, el hecho de que la anoxia es una alteración frecuente en la clínica y el interés en el mejor conocimiento de las poblaciones situadas en regiones elevadas han motivado diversos estudios de la policitemia de altura en años recientes. Revisaremos en forma breve y esquemática sus principales aspectos utilizando en parte datos obtenidos en investigaciones realizadas en la Cátedra de Fisiopatología, Facultad de Medicina, Lima, y en los laboratorios de Morococha del Instituto de Biología Andina,† dejando para la reunión de mesa redonda la oportunidad de ampliar la discusión de algunos de los tantos interrogantes todavía presentes.

1. *Influencia del grado y duración de la anoxia*

Al igual de lo que ocurre en otras alteraciones o modificaciones en el organismo humano, cuando es sometido a la influencia de un estado de anoxia, el sistema hematopoyético presenta gran variabilidad en su reacción a tal factor. Pero en general la producción e intensidad de la policitemia resultante tiene una evidente relación con el grado y duración de la anoxia. El frecuente desconocimiento de este hecho ha originado cierta confusión en la literatura.

Si la anoxia es de corta duración, tal como corresponde a las primeras horas de exposición a un ambiente de baja presión en la altura o en cámaras de descompresión, la respuesta policitémica es particularmente variable y aun puede no ocurrir.[2]

Las alteraciones hematológicas en el inicio de tal exposición incluyen (Figura 1) a menudo una moderada elevación de los leucocitos circulantes que afecta en nuestra experiencia a los neutrófilos segmentados.[2]

En los sujetos nativos, habitantes permanentes en regiones elevadas, y en

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† Algunas de estas observaciones han sido ya publicadas y otras lo serán dentro de un breve tiempo. En su realización han participado los siguientes miembros de estas instituciones: (a)—Características de la policitemia en la aclimatación natural: Drs. Alberto Hurtado, César Merino, César Reynafarge y Ernesto Delgado. (b)—Policitemia correspondiente a una residencia temporal en la altura: Drs. César Reynafarge, Alberto Hurtado y César Merino. (c)—Biopsias de la médula ósea: Drs. César Merino y César Reynafarge. (d)—Procesos de destrucción globular: Drs. César Merino, César Reynafarge, Rodolfo Lozano, Ernesto Delgado, Srs. Celestino Sánchez y Julio Muñóz. (e)—Afinidad de la hemoglobina por oxígeno: Drs. Humberto Aste Salazar, Alberto Hurtado, Tulio Velasquez y Baltazar Reynafarge.

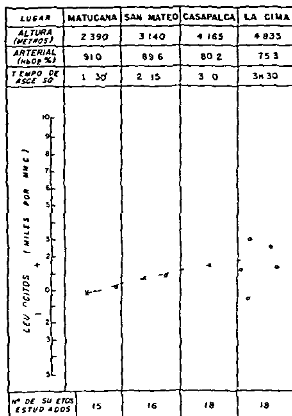


FIG 2 —Cambios en los leucocitos de la sangre expresados en miles por mm³ en varios grupos de sujetos normales estudiados durante las dos primeras horas de exposición a diversas alturas

ciones pulmonares el nivel de policitemia que fue más intenso mientras mayor era la anoxemia cesó de presentar esta relación a saturaciones arteriales inferiores a 60 %. En estos casos se observó una disminución de la policitemia que afectaba proporcionalmente más a la cantidad de hemoglobina que al numero de hematíes (Figura 4)

Estos hallazgos sugieren que el factor anoxémico pierde su carácter estimulante y se torna más bien deprimente de la función eritropoyética cuando alcanza niveles de saturación arterial alrededor de 60 % correspondiente a una altura aproximada de 6 000 metros (20 700 pies) y que esta acción afecta principalmente a la producción de hemoglobina en comparación con el estroma eritrocítico La ausencia de un mayor grado de bilirrubinemia en estos casos parece indicar que no se trata de una acentuación en los procesos de destrucción globular

Cuando la anoxia tiene un carácter intermitente la frecuencia e intensidad de la respuesta policitémica guarda también una relación general con su grado y duración En el estudio que hemos efectuado de dos grupos de sujetos Grupo I

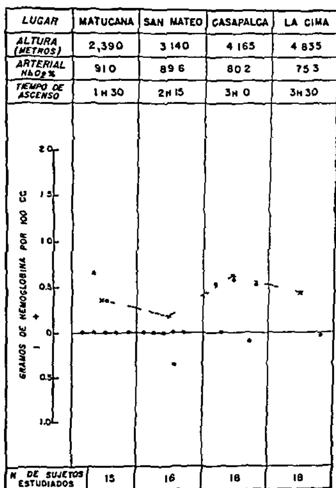


FIG 1 —Cambios en la hemoglobina de la sangre expresados en gramos por 100 cc en varios grupos de sujetos normales estudiados durante las dos primeras horas de exposición a diversas alturas

consecuencia sometidos a la acción constante de cierto grado de anoxemia, el nivel de policitemia aunque variable tiene una relación inversa y proporcional más precisa con el grado de saturación arterial al oxígeno (Figura 2) El efecto estimulante de la anoxemia sobre la actividad eritropoyética parece tener un límite, que no depende de la capacidad anatómica de respuesta de los órganos responsables de la formación de los elementos sanguíneos (Figura 3) Talbott en 1936 observó en los miembros de una expedición a los Andes de Chile que el mayor grado de policitemia ocurrió a una altura de 5 340 metros (17 500 pies) con una HbO2 % arterial de 76 2 % En un ascenso posterior a 6 140 metros (20 140 pies) que redujo la saturación a [illegible] % la hemoglobina circulante disminuyó En el estudio de un grupo de 82 sujetos con Neumoconiosis realizado por nosotros en Oroya a 3 730 metros (12 240 pies) enfermos en los que el grado de anoxemia estaba anormalmente reducido a consecuencia de las altera

Personal de una línea aérea comercial que opera en Sud América, con un promedio individual de 60–80 horas de vuelo mensual y Grupo II Personal de un ferrocarril que une Lima al nivel del mar con Oroya a 3 730 metros (12 240 pies) de altura y que con excepción de una vacación anual de un mes duerme alternativamente en Lima y en Oroya y viaja diariamente por una vía que alcanza una elevación de 4 740 metros (15 550 pies) observamos alteraciones policitémicas mas frecuentes en el segundo grupo Entre el personal de vuelo un 23 % presentaron concentraciones de hemoglobina superiores a 17 50 gramos por 100 cc de sangre mientras que este hallazgo fué verificado en un 69 % del Grupo II En este ultimo grupo el volumen total circulante de los hematíes (expresado en cc por kilo de peso corporal) estuvo elevado en un 64 % de los sujetos Los valores correspondientes a los reticulocitos y bilirrubina plasmatica de tipo indirecto presentaron elevaciones proporcionales

2 *Policitemia en la aclimatacion natural a la altura*

Los estudios no muy numerosos hechos en residentes permanentes en las grandes alturas se refieren en su gran mayoría solo a los valores de hematíes hemoglobina y leucocitos Alhalel[4] Chiodi,[5] Huff, Lawrence y otros[6] han ampliado estas observaciones en anos recientes

Nuestras investigaciones sobre aclimatación natural a la altura se han realizado principalmente en nativos indígenas residentes permanentes en Morococha localidad situada en la region central andina del Peru a una elevacion de 4 540 metros (14 900 pies) y ellas incluyen algunas de carácter hematologico las que *presentaremos en forma esquematica para ilustrar las caracteristicas de la* policitemia correspondiente a la aclimatacion natural a un medio ambiente de baja presion Los promedios de presión barométrica tensión parcial del oxígeno en el aire alveolar y saturacion de la sangre arterial en estos sujetos son 446 mmHg 50 mmHg y 80 % respectivamente indicando la presencia de un grado moderadamente severo de anoxia anoxica o anoxemia Con fines comparativos observaciones similares han sido llevadas a cabo en sujetos adultos normales residentes en Lima ciudad situada a una altura de 150 metros (500 pies), la que puede considerarse como nivel del mar desde el punto de vista fisiologico ambiental

En Morococha se aprecia un definido aumento en el numero de hematíes, concentración de hemoglobina y volumen de hematíes por 100 cc de sangre (hematocrito) El numero de leucocitos y plaquetas no varía En la formula leucocitaria expresada en numero absoluto de sus diversos componentes, la unica diferencia estadísticamente significativa es un ligero aumento de linfocitos en la altura Aunque la variabilidad observada en Morococha en los valores de hematíes y hemoglobina es mayor que la correspondiente al nivel del mar no hemos hallado las marcadas *fluctuaciones senaladas por Chiodi*[5] en estudios realizados a una altura casi idéntica a la nuestra Esta diferencia posiblemente se debe al hecho de ser nuestros sujetos nativos de raza india nacidos y desarrollados en un medio ambiente elevado en tanto que los estudiados por Chiodi son residentes temporales aunque en su mayoría con varios anos de residencia (Cuadro 1)

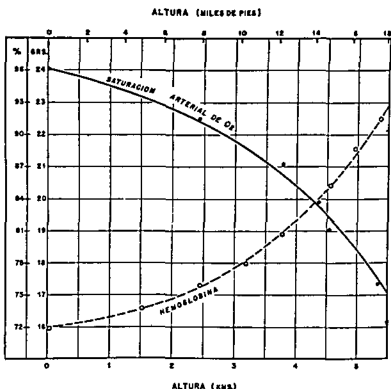


FIG 3 —Relacion entre los valores medios de HbO2 % arterial y gramos de Hb por 100 cc en sujetos nativos residentes a diversas alturas

% GM
78 27
76 26
74 25
72 24
70 23
68 22
66 21
64 20
62 19
60 18
Hb
Ht
34
33
32
31
30
29
Ht/Hb x 100
+840 839-720 719 600 -600
ART HbO2 %

FIG 4 —Valores promedios de hemoglobina hematocrito y concentracion de Hb globular en casos de Neumoconiosis estudiados a una altura de 3 30 metros y agrupados de acuerdo con el nivel de HbO2 % arterial

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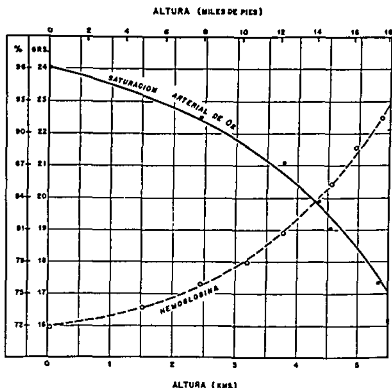


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Hb
Ht
34
33
32
31
30
29
Ht/Hb x 100
+ 84.0
83.9 72.0
71.9 60.0
- 60.0
ART HbO2 %

FIG 4 —Valores promedios de hemoglobina hematocrito y concentracion de Hb globular en casos de Neumoconiosis estudiados a una altura de 3 750 metros y agrupados de acuerdo con el nivel de HbO2 % arterial

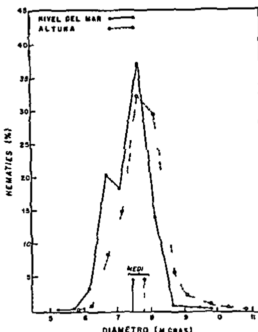


FIG 5 —Diámetro de los hematíes circulantes (Curva de Price Jones) en 100 sujetos normales residentes en Lima al nivel del mar (29 500 hematíes medidos) y en 32 nativos residentes en Morococha a 4 540 metros de altura (5 100 hematíes medidos)

La policitemia de los residentes en la altura es de tipo absoluto Hay un aumento en el volumen total de sangre circulante a expensas de la masa globular El volumen plasmático está reducido (Figura 6) Estos hallazgos concuerdan con los previos de Lippmann[7] en Suiza y Lozoya Solís[8] en Méjico Recientemente, Lawrence y otros[9] empleando fósforo radioactivo hallaron en once nativos *residentes en Morococha un aumento moderado en el volumen de hematíes* y un volumen normal de plasma La diferencia entre sus valores absolutos y los nuestros mas altos puede ser atribuída a la ya demostrada discrepancia que existe entre los resultados obtenidos mediante el empleo de isótopos radioactivos y colorantes[10] para la determinación del volumen de sangre circulante En la mayoría de nuestras observaciones hemos usado el Azul de Evans en unos pocos hace varios anos el Rojo Vital Brillante

En la altura el grado de reticulocitosis relativa y absoluta es mayor que al nivel del mar aunque es de notar que existe una marcada variacion individual y que en muchos sujetos ésta se encuentra entre límites normales Hay aumento en el hierro plasmático y en las entroporfirinas * a juzgar por un corto numero de determinaciones que hemos realizado (Cuadro 3) Los valores medios en

* Las técnicas empleadas para estas determinaciones fueron las siguientes Hierro C V Moore J of Clin Inv 16 613 1937 Eritroporfirinas—Grinstein M y Watson C J The J of Biol Chem 1 7 6 5 1943

Cuadro 1 — *Valores medios hematologicos en sujetos normales residentes en Lima al nivel del mar y en nativos de raza india residentes en Morococha a 4 540 metros de altura*

	Lima (150 mts)		Morochoba (4,540 mts)	
	N Sujetos	Media ± E S	N Sujetos	Media ± E S
Hematíes (mill mc)	250	5 11 ± 0 02	83	6 44 ± 0 09
Hemoglobina (gr 100 cc)		15 64 ± 0 05		20 13 ± 0 22
Hematocrito (%)		46 6 ± 0 15		59 5 ± 0 68
Plaquetas (mil mc)		406 ± 15	51	454 ± 24
Leucocitos (mil mc)	140	6 68 ± 0 10	72	7 04 ± 0 19
Neutr a bast (mil mc)		0 24 ± 0 02		0 27 ± 0 02
Neutr segm (mil mc)		3 51 ± 0 09		3 72 ± 0 15
Neutr tot (mil mc)		3 74 ± 0 09		3 95 ± 0 16
Eosinófilos (mil mc)		0 28 ± 0 02		0 20 ± 0 02
Basofilos (mil mc)		0 — 0 3		0 — 0 2
Monocitos (mil mc)		0 46 ± 0 02		0 39 ± 0 02
Linfocitos (mil mc)		2 16 ± 0 05		2 47 ± 0 11

Las constantes globulares de tamaño forma y contenido de hemoglobina de los hematíes circulantes son *practicamente idénticas al nivel del mar y* en la altura a excepcion de una ligera desviacion a la derecha en la curva de Price-Jones en este ultimo lugar indicando una mayor frecuencia de hematíes grandes (Figura 5) Hay diferencia estadística significativa entre los valores medios de diametro globular

Si tomamos en cuenta que en los sujetos normales al nivel del mar existe una relacion inversa entre el numero de hematíes por mm^3 y el volumen medio globular y que a una numeración elevada de mas de 5 50 millones corresponde un valor medio de 86 3 micras[3] tenemos que el promedio de 92 8 micras[3] obtenido en la altura para una numeracion de 6 44 millones indica comparativamente una macrocitosis relativa y moderada (Cuadro 2)

Cuadro 2 — *Valores medios de las constantes correspondientes al tamaño forma y contenido de Hb de los hematíes circulantes en Lima al nivel del mar y en Morococha a 4 540 metros de altura*

Constantes globulares	Lima (150 mts)		Morococha (4 540 mts)	
	N Sujetos	Media ± E S	N Sujeto	Media ± E S
Volumen medio (micr ³)	250	91 2 ± 0 30	83	92 8 ± 0 41
Diámetro medio (micr)	130	7 48 ± 0 01	32	7 74 ± 0 01
Grosor medio (micr)		2 09 ± 0 01		2 05 ± 0 02
Area de sup media (micr ²)		137 ± 0 22		145 ± 1 18
Indice esferocitico		0 28 ± 0 01		0 27 ± 0 002
Hemoglobina media (micr g)	250	30 9 ± 0 11	83	31 5 ± 0 31
Conc media de Hb (%)		33 8 ± 0 08		33 9 ± 0 15

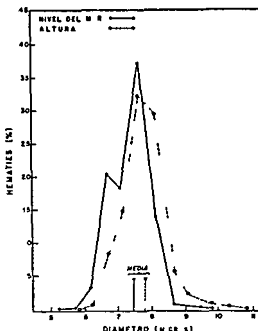


FIG 5 —Diámetro de los hematíes circulantes (Curva de Price Jones) en 100 sujetos normales residentes en Lima al nivel del mar (29,500 hematíes medidos) y en 32 nativos residentes en Morococha a 4,540 metros de altura (5 100 hematíes medidos)

La policitemia de los residentes en la altura es de tipo absoluto Hay un aumento en el volumen total de sangre circulante a expensas de la masa globular El volumen plasmatico esta reducido (Figura 6) Estos hallazgos concuerdan con los previos de Lippmann[7] en Suiza y Lozoya Solís[8] en Méjico Recientemente Lawrence y otros[9] empleando fosforo radioactivo hallaron en once nativos residentes en Morococha un aumento moderado en el volumen de hematíes y un volumen normal de plasma La diferencia entre sus valores absolutos y los nuestros mas altos puede ser atribuída a la ya demostrada discrepancia que existe entre los resultados obtenidos mediante el empleo de isotopos radioactivos y colorantes[10] para la determinación del volumen de sangre circulante En la mayoria de nuestras observaciones hemos usado el Azul de Evans en unos pocos hace varios anos el Rojo Vital Brillante

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CUADRO 1 — *Valores medios hematologicos en sujetos normales residentes en Lima al nivel del mar y en nativos de raza india residentes en Morococha a 4 540 metros de altura*

	Lima (150 mts)		Morococha (4 540 mts)	
	N Sujetos	Media ± E S	N Sujetos	Media ± E S
Hematíes (mill/mc)	250	5.11 ± 0.02	83	6.44 ± 0.09
Hemoglobina (gr/100 cc)		15.64 ± 0.05		20.13 ± 0.22
Hematocrito (%)		46.6 ± 0.15		59.5 ± 0.68
Plaquetas (mil/mc)		406 ± 15	51	455 ± 24
Leucocitos (mil/mc)	140	6.68 ± 0.10	72	7.04 ± 0.19
Neutr. abast. (mil/mc)		0.24 ± 0.02		0.27 ± 0.02
Neutr. segm. (mil/mc)		3.51 ± 0.09		3.42 ± 0.15
Neutr. tot. (mil/mc)		3.74 ± 0.09		3.95 ± 0.16
Eosinófilos (mil/mc)		0.28 ± 0.02		0.20 ± 0.02
Basófilos (mil/mc)		0 – 0.3		0 – 0.2
Monocitos (mil/mc)		0.46 ± 0.02		0.39 ± 0.02
Linfocitos (mil/mc)		2.16 ± 0.05		2.47 ± 0.11

Las constantes globulares de tamaño, forma y contenido de hemoglobina de los hematíes circulantes son practicamente idénticas al nivel del mar y en la altura a excepcion de una ligera desviacion a la derecha en la curva de Price Jones en este ultimo lugar indicando una mayor frecuencia de hematíes grandes (Figura 5). Hay diferencia estadística significativa entre los valores medios de diametro globular.

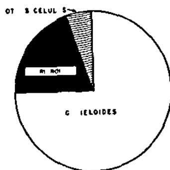
Si tomamos en cuenta que en los sujetos normales al nivel del mar existe una relación inversa entre el numero de hematíes por mm³ y el volumen medio globular [2] y que a una numeracion elevada de mas de 5.50 millones corresponde un valor medio de 86.3 micras³ tenemos que el promedio de 92.8 micras³ obtenido en la altura para una numeración de 6.44 millones indica comparativamente una macrocitosis relativa y moderada (Cuadro 2).

CUADRO 2 — *Valores medios de las constantes correspondientes al tamaño, forma y contenido de Hb de los hematíes circulantes en Lima al nivel del mar y en Morococha a 4 540 metros de altura*

Constantes globulares	Lima (150 mts)		Morococha (4 540 mts)	
	N Sujetos	Media ± E S	N Sujetos	Media ± E S
Volumen medio (micr³)	250	91.2 ± 0.30	83	92.8 ± 0.??
Diámetro medio (micr.)	130	7.48 ± 0.01	32	7.74 ± 0.01
Grosor medio (micr.)		2.09 ± 0.01		2.08 ± 0.02
Area de sup. media (micr²)		137 ± 0.22		145 ± 1.18
Indice esferocítico		0.28 ± 0.01		0.27 ± 0.009
Hemoglobina media (micr g)	250	30.9 ± 0.11	83	31.5 ± 0.31
Conc. media de Hb (%)		33.8 ± 0.08		33.9 ± 0.15

CITOLOGIA MEDULAR

	A NIVEL DEL MAR	ALTURA
CÉLULAS MIELOIDES	75 0 %	40 9 %
CELULAS ERITROIDES	20 0 %	55 6 %
OTRAS CELULAS	5 0 %	3 5 %



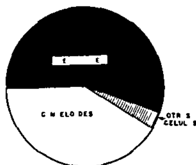
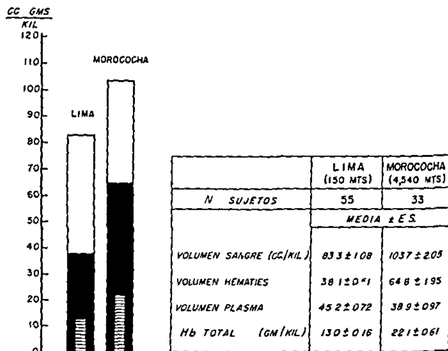


FIG 7 —Relación eritroide/mieloide en biopsias medulares obtenidas en sujetos normales residentes en Lima al nivel del mar y en nativos residentes en Cerro de Pasco a 4 390 metros de altura Tomada de Merino y Reynafarge [14]

presentaron valores de bilirrubina total por encima de 1 mg por 100 cc mientras que este hecho fué verificado en el 29 % de los residentes en Morococha Hallazgos similares han sido comunicados por Delgado [12]

Merino y Reynafarge [14] en 1949 llevaron a cabo el estudio de biopsias medulares en 16 sujetos normales residentes nativos en Cerro de Pasco a una altura de 4 390 metros (14 240 pies), o sea ligeramente menor a la correspondiente a Morococha y señalaron la existencia de una hiperplasia eritroide de tipo normoblástico en el tejido de la medula ósea (Figura 7) La relacion celular eritroide/mieloide fué encontrada invertida Indicaron también que los megacariocitos al igual que los mielocitos no estaban aumentados y que las características cualitativas de maduracion fueron halladas normales en ambas series eritroide y mieloide Recientemente Huff Lawrence y otros [6] en un menor numero de observaciones hechas en Morococha han confirmado la existencia de esta hiperplasia eritroide en residentes en la altura aunque el grado encontrado fué menor a lo señalado por Merino y Reynafarge La hiperactividad eritropoyetica en los sujetos aclimatados ha sido también comprobada por Lawrence y otros[9] en el estudio del metabolismo del hierro mediante la administracion de isotopos radioactivos



	LIMA (150 MTS)	MOROCOCHA (4,540 MTS)
N SUJETOS	55	33
	MEDIA ± E.S.	
VOLUMEN SANGRE (CC/KIL)	83 3 ± 1 08	103 7 ± 2 05
VOLUMEN HEMATIES	38 1 ± 0 41	64 8 ± 1 95
VOLUMEN PLASMA	45 2 ± 0 72	38 9 ± 0 97
Hb TOTAL (GM/KIL)	13 0 ± 0 16	22 1 ± 0 61

FIG 6 —Valores promedios de volumen total de sangre circulante y sus componentes en sujetos normales residentes en Lima al nivel del mar y en nativos residentes en Morococha a 4 540 metros de altura

Lima corresponden a los que se han establecido como normales[11, 1] En el caso del hierro plasmatico la concentracion promedio en la altura esta entre los límites de variacion encontrados en sujetos normales residentes al nivel del mar pero excede significativamente la encontrada en Lima en nuestra serie de sujetos El valor medio de las eritroporfirinas en Morococha esta por encima del límite que Watson[12] considera como normal La bilirrubina plasmática se encuentra frecuentemente elevada en la altura y el aumento esta hecho a expensas de la fraccion indirecta En Lima un 17% de los sujeto estudiados

CUADRO 3 —*Valores promedios de algunas caracteristicas hematologicas en sujetos normales residentes en Lima al nivel del mar y en Morococha a 4 540 metros de altura*

	Lima (150 mts)		Morococha (4 540 mt)	
	N Sujeto	Med ± E S	N Sujeto	Med ± E S
Reticulocitos (%) (mil mmc)	250	0 4 ± 0 02 17 9 ± 1 00	83	1 0 ± 0 07 45 5 ± 4 43
Hierro plasmatico (μg %)	9	86 ± 5 2	10	135 ± 31
Protoporfirinas (μg %)	10	35 0 ± 6 33	9	66 3 ± 15
Bilirrubina plasmática	150		67	
Total (mg %)		0 76 ± 0 03		1 28 ± 0 11
Directa (mg %)		0 33 ± 0 01		0 36 ± 0 02
Indirecta (mg %)		0 42 ± 0 02		0 92 ± 0 09

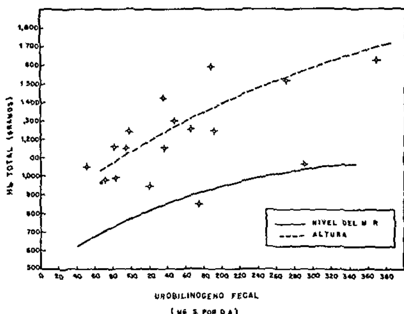


Fig 8 —Relación entre la cantidad total de Hb circulante y la excreción fecal de urobilinógeno en residentes normales en Lima al nivel del mar y en nativos residentes en Morococha a 4,540 metros de altura Nota Este diagrama corresponde originalmente a las *observaciones de Merino*[16] *a las que hemos agregado las que hemos realizado recientemente* (no publicadas)

Tiene a nuestro juicio importancia fundamental señalar el hecho de que el estímulo del factor anóxico es específico para la actividad eritropoyética no afectando la formación de leucocitos y plaquetas y que la respuesta de la medula ósea en su hiperactividad eritropoyética se desenvuelve dentro de un ritmo cualitativamente normal

3 *Desarrollo de la policitemia de altura*

(A) *Durante las primeras horas y días de exposición a un medio ambiente de baja presión* Ya hemos señalado que durante las primeras horas después de la llegada a un lugar elevado ocurre en la mayoría de los sujetos un aumento moderado y variable en la hemoglobina circulante Paralelamente se elevan las proteínas del plasma y a las 24–48 horas se aprecia el inicio de una reticulocitosis que en los días siguientes alcanza valores relativamente altos (Figura 9) Al mismo tiempo la hemoglobina sigue aumentando

En determinaciones de volumen total de sangre circulante hemos encontrado al inicio de la exposición una disminución *en el volumen plasmático* (±10% menor que antes del ascenso) en la mayoría de los sujetos acompañada por un aumento en la masa globular (±12%) (Figura 10) Si la permanencia en la altura se prolonga por varios días y semanas el aumento de los *hematíes* se hace

CUADRO 4.—*Valores medios de Hb total en la sangre, cantidad total de bilirrubina en el plasma y excreción de urobilinógeno y coproporfirinas en sujetos normales residentes en Lima al nivel del mar y en Morococha a 4 540 metros de altura*

	Lima (150 mt.)		Morococha (4 540 mts)	
	N Sujetos	Media ± E.S.	N Sujetos	Media ± E.S.
Hemoglobina total (gms)	19	*808 ± 28*	*16*	1 176 ± 48
Urobilinógeno* (mgm/día)		*123 ± 21*		156 ± 22
Indice hemolítico†		15.1 ± 2.25		13.5 ± 1.76
Bilirrubina total (mgm)		27 ± 4.50		63 ± 16
Coproporfirinas orina (µg/día)	10	72 ± 6.43	6	105 ± 23
Coproporfirinas heces (µg/día)		*333 ± 42*		1 139 ± 141

* En heces y orina

† $\frac{\text{Urobilinogeno} \times 100}{\text{Hb total}}$

La mayor producción de hematíes y hemoglobina en la altura y su mayor cantidad en la sangre circulante son balanceados por un proceso de destrucción globular más activo. Merino[15] en 1950 halló un aumento en la excreción de urobilinógeno fecal en un grupo de residentes nativos en Morococha. La cantidad excretada no excedió, sin embargo, los límites considerados como normales al nivel del mar. En estudios recientes en un mayor número de residentes hemos confirmado estos hallazgos y los resultados de ambas investigaciones están sumarizados en el Cuadro 4. La relación entre la cantidad total de hemoglobina circulante y urobilinógeno excretado (representada por el llamado *Indice Hemolítico*) es prácticamente idéntica al nivel del mar y en la altura (*Figura 8*). La bilirrubina plasmática total (calculada utilizando la cifra de volumen de plasma y concentración de bilirrubina en 100 *cc*) *está francamente* aumentada en la altura. Hemos también constatado una marcada elevación en la eliminación de coproporfirinas en las heces y orina.*

Reissmann y otros[16] han encontrado un aumento en la producción de pigmentos biliares en perros sometidos a una baja presión en cámaras.

En resumen, la policitemia correspondiente a la aclimatación natural a una altura de 4 540 metros (14 900 pies) es de tipo absoluto, con aumento del volumen de hematíes y disminución menos intensa del plasma y está asociada a una hiperactividad eritropoyética balanceada, a su vez, por un aumento proporcional en los procesos de destrucción globular. Estas últimas características explican, al parecer, la mayor tasa de eritroporfirinas, hierro plasmático y el aumento en la eliminación de pigmentos por vías intestinal y renal. La elevación de la bilirrubina plasmática, de tipo indirecto, tiene posiblemente igual explicación, aunque puede estar también influenciada por una menor capacidad excretora del hígado como consecuencia de la anoxia.[17, 18]

* Para la determinación de las coproporfirinas se utilizó el siguiente método: Schwartz, S., Hawkinson, V., Cohen, S. y Watson, C. J.—The J. of Biol. Chem. *168*: 133, 1947.

Fig 10 —Volumen total de sangre circulante (hematíes y plasma) determinado en dos grupos de sujetos I—Dentro de las dos primeras horas después de la llegada a una altura de 4 540 metros y II—Después de 2-6 días de permanencia en esta altura Los valores obtenidos en la altura (Alt) son comparados con los correspondientes a previas determinaciones hechas al nivel del mar (N M) antes del ascenso

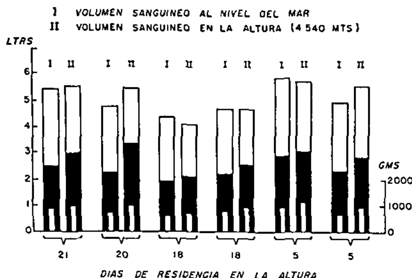


Fig 11 —Volumen total de sangre circulante (plasma hematíes y hemoglobina) en seis sujetos normales de pués de una residencia de 5 21 días en Morococha a 4 540 metros de altura Tomada de Merino

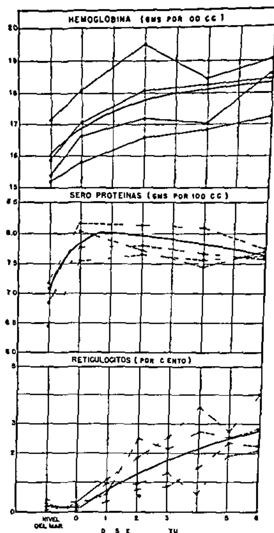


FIG. 9.—Curvas de hemoglobina (gramos por 100 cc de sangre), sero proteínas (gramos por 100 cc) y reticulocitos (% de hematíes) en cuatro sujetos normales estudiados en el momento de la llegada y durante una permanencia de seis días a una altura de 4 540 metros

más marcado (±22%) (Figura 11). La reticulocitosis persiste durante esta acentuación gradual de la policitemia pero no hay variación en el número de leucocitos y plaquetas[2, 16]. Reissmann[19] en observaciones realizadas en perros expuestos en cámaras a una baja presión ha hallado también una disminución en el volumen del plasma y una elevación en el volumen de los hematíes, modificaciones ambas que fueron evidentes a los dos días de exposición.

Merino[15] ha indicado que este período inicial de permanencia en la altura está también caracterizado por un aumento en la excreción de urobilinógeno.

Las observaciones realizadas por Lawrence y otros[9] en sujetos normales durante los primeros días de estadía a una altura idéntica coinciden con las nuestras en lo concerniente a la disminución del volumen plasmático, pero estos

LITROS

GRUPO I

SUJETOS 1 2 3 4 5 6

HORAS DESPUES DE LLEGADA 1 2 1 2 1 2

LITROS

GRUPO II

SUJETOS 1 2 3 4 5

DIAS DESPUES DE LLEGADA 2 4 4 6 6

Fig 10 —Volumen total de sangre circulante (hematíes y plasma) determinado en dos grupos de sujetos I—Dentro de las dos primeras horas después de la llegada a una altura de 4 540 metros y II—Después de 2-6 días de permanencia en esta altura Los valores obtenidos en la altura (Alt) son comparados con los correspondientes a previas determinaciones hechas al nivel del mar (N M) antes del ascenso

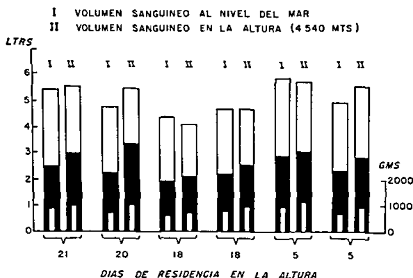


Fig 11 —Volumen total de sangre circulante (plasma hematíes y hemoglobina) en seis sujetos normales después de una residencia de 5 21 días en Morococha a 4 540 metros de altura Tomada de Merino [15]

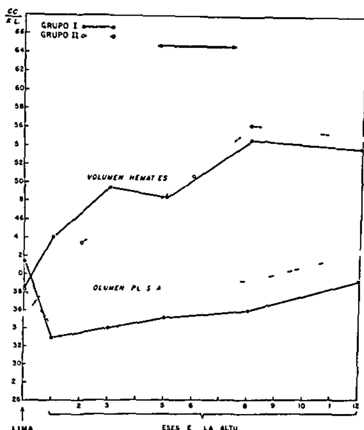


FIG 12 —Volumen total de hematíes y plasma (en cc por kilo de peso corporal) en dos grupos de cinco sujetos normales cada uno estudiados durante un año de residencia constante en Morococha a 4 540 metros de altura La línea ↔ corresponde al promedio de volumen de hematíes encontrado en nativos residentes a esta altura

investigadores no encontraron aumento sino mas bien una ligera disminución en el volumen de los hematíes En estos sujetos observaron una mayor utilización de hierro radioactivo inyectado

B *Durante una permanencia prolongada en la altura* Con el objeto de apreciar los procesos de aclimatación adquirida a la altura hemos estudiado recientemente un grupo de diez sujetos adultos normales durante un año de residencia ininterrumpida en Morococha a una altura de 4 540 metros (14 900 pies) Las observaciones (por publicarse) realizadas mensualmente incluyeron algunas de carácter hematológico El volumen total de hematíes (en cc por kilo de peso corporal) acusó un aumento progresivo alcanzando su máximo valor al octavo mes tiempo en el que la curva se estabilizó (Figura 12) Es de notar que no fué alcanzado durante el año el nivel de policitemia correspondiente a los nativos residentes Igual hecho hemos constatado en el estudio de otros sujetos no nativos aunque con residencia de varios años en la altura El volumen del plasma expresado en igual forma descendió durante los dos primeros meses elevándose después en los siguientes El hierro plasmático y las eritroporfirinas acusaron

una tendencia a la elevación, aunque con definidas fluctuaciones durante este período La concentración de bilirrubina prácticamente no varió y las plaquetas y *leucocitos se mantuvieron dentro de límites normales* La fórmula leucocitaria reveló una linfocitosis relativa durante los dos primeros meses Biopsias medulares hechas en algunos de los sujetos durante el año mostraron una hiperplasia *del tejido eritroide menos marcada que la hallada en nativos residentes, y con* caracteres normales de la serie mieloide

El aumento gradual de la hemoglobina total circulante que también alcanzó *su máximo valor al octavo mes estuvo acompañado por un aumento moderado* en la excreción de urobilinógeno (Figura 13) El índice hemolítico no presentó

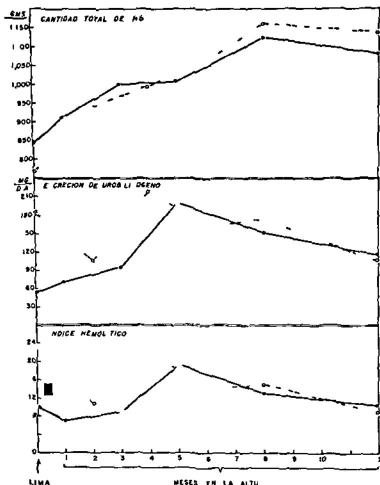


FIG 13 —Cantidad total de Hb en la sangre (gramos) excreción de urobilinógeno en heces y orina (mg/día) e índice hemolítico (urobilonogeno × 100/hemoglobina) en dos grupos de sujetos normales estudiados durante un año de residencia constante en Morococha a 4,540 metros de altura ■ corresponde a los valores promedios de índices hemolíticos encontrados al nivel del mar

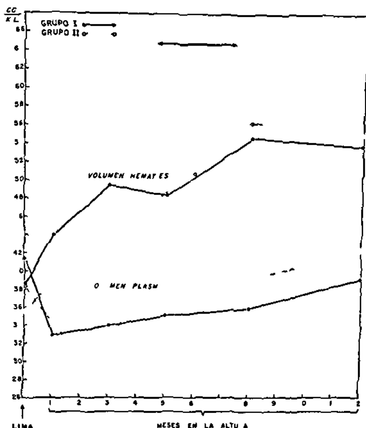


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geno hay sin embargo cierta discusión sobre el mecanismo en virtud del cual ejerce tal acción. Algunos investigadores han puesto en duda la acción directa sobre la médula ósea y han señalado en animales hechos anóxicos por anemia o por exposición a una concentración de oxígeno baja en cantidad o en presión la existencia en el suero de substancias capaces de estimular la eritropoyesis[31,32]. El mecanismo humoral de la policitemia de la anoxia ha sido recientemente investigado por Reissmann[33] en ratas parabióticas en las que solo uno de los dos animales fué sometido a la inhalación de bajas concentraciones de oxígeno. Reissmann observó el desarrollo de policitemia tanto en el animal anóxico como en el animal respirando aire este último unido al primero por medio de conexiones vasculares al nivel de la red capilar.

Las investigaciones que hemos citado, las que a nuestro juicio no constituyen todavía una evidencia concluyente, especialmente en lo que respecta a su aplicación a los fenómenos que ocurren en el hombre en igual condición, arroja sin embargo un interrogante sobre el mecanismo en virtud del cual el factor anóxico ejerce su acción estimulante sobre la actividad eritropoyética: por vía humoral, con intervención de factores neuro-endocrinos o directamente sobre el tejido hemopoyético? En favor de este último concepto están las recientes observaciones de Schwartz y Stats[34] quienes han encontrado en casos de anoxia anóxica o anoxemia una disminución en la saturación de la sangre obtenida de la médula ósea por punción, y las de Hecht y Samuels[35] quienes en determinaciones del contenido de oxígeno en biopsias de la médula ósea esternal hallaron un paralelismo con los valores correspondientes a la sangre venosa mixta y por lo tanto una disminución en dicho contenido en casos de anoxia arterial y policitemia, observaciones todas estas que sugieren una probable acción directa sobre el tejido hemopoyético.

Por último debemos mencionar que no tenemos todavía información acerca de otra característica importante de la policitemia de los sujetos que viven en un lugar elevado. Nos referimos a la duración de la vida de los hematíes. Actualmente Lawrence y Berlin de la Universidad de California en colaboración con Reynafarje de nuestro laboratorio están estudiando este problema mediante el uso de fierro radioactivo. En perros policitémicos por anoxia producida en *cámaras* Reissmann y otros[18] no han observado modificación en la vida de los glóbulos rojos.

3. La policitemia de altura como mecanismo adaptativo

La policitemia es considerada justificadamente como uno de los tantos mecanismos adaptativos que el organismo pone en juego en la altura para compensar los efectos de la anoxia. Transfusiones de sangre han sido utilizadas experimentalmente para mejorar la tolerancia a una baja presión ambiental[36].

Los efectos favorables de la policitemia influyen en dos procesos: (A) En el transporte del CO_2, pues los hematíes toman aproximadamente 40% de este gas a su paso por los tejidos y proporcionan la enzima respiratoria anhidrasa carbónica que asiste en la conversión del CO_2 a bicarbonato y en su liberación a nivel pulmonar[37] y (B) En el transporte del O_2 aumentando su contenido en la

valores anormales, con excepción del 4° y 5° mes, en que se observó un ligero aumento. Estas observaciones indican que a la hiperactividad eritropoyética correspondió una elevación proporcional en los procesos de destrucción globular.

La eliminación de coproporfirinas, en heces y orina, aumentó fuertemente durante el año, alcanzando sus valores más elevados alrededor del 4°–5° mes de permanencia en la altura. La excreción al fin del año no bajó al nivel previamente observado en Lima antes del ascenso.

4. Mecanismos de producción de la policitemia de altura

A. *En la policitemia inicial.* Por lo menos dos mecanismos parecen intervenir en el aumento inmediato de los hematíes y hemoglobina durante las primeras horas de exposición a un ambiente elevado: hemoconcentración y liberación de sangre almacenada. La pérdida de agua del plasma circulante con la consecuente hemoconcentración ha sido verificada como ya lo hemos indicado de manera indirecta por el aumento de sero proteínas y directamente por la determinación del volumen plasmático. Asmussen y Nielsen [6] mediante estudios de balance acuoso han hallado una pérdida de agua del plasma y de los tejidos en sujetos expuestos a una baja presión en cámaras. La liberación de hematíes almacenados en diversos órganos: médula ósea, hígado, bazo y quizás otros, bajo la influencia de un estado de anoxia aguda, es un proceso más discutido pero el que puede explicar satisfactoriamente el aumento absoluto en la masa globular y hemoglobina observado por la mayoría de los investigadores durante las primeras horas de exposición, ya que mecanismos de redistribución de sangre en el sistema vascular no han sido comprobados.[2] El rol del bazo ha sido especialmente debatido. Señalado este órgano por Barcroft [1] y en numerosas observaciones aparecidas en la literatura como el responsable principal de la liberación de hematíes bajo la acción simpático adrenal puesta en juego por el factor anóxico, esta función le fue negada por Ebert y Stead.[2] Sin embargo recientes observaciones [3,2] y de manera especial las de Kramer y Luft [6] parecen confirmar que este órgano es en realidad un reservorio de glóbulos rojos los que son liberados en condiciones en que el organismo necesita aumentar su capacidad de transporte del oxígeno, tal como sucede en una condición de anoxia aguda.

B. *En la policitemia permanente.* Clásicamente se ha aceptado que la tensión del oxígeno en la sangre circulante constituye un factor regulador de la actividad eritropoyética. En favor de esta opinión se señala la hiperactividad en esta función y la policitemia resultante durante la exposición prolongada o permanente a un ambiente elevado, ambiente en el cual la tensión disminuye por razón de la baja presión parcial de oxígeno en el aire inspirado, y los estados de policitemia secundaria o eritrocitosis en enfermos con alteraciones pulmonares y circulatorias en quienes se altera la adquisición de dicho gas a nivel alveolar. Hay también evidencia de lo contrario, es decir, que una elevación en la tensión del oxígeno inspirado deprime la actividad eritropoyética tanto en sujetos normales como en patológicos.[7]

Aceptada la acción estimulante eritropoyética de la menor tensión del oxí

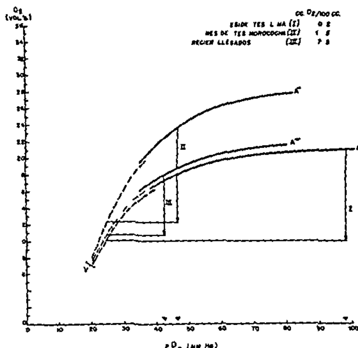


Fig 15 —Cálculo teórico de la cantidad de O_2 liberada de la sangre a un pO_2 de 25 mm Hg en tres grupos de sujetos I—Sujetos normales residentes en Lima al nivel del mar II—Nativos residentes en Morococha a 4 540 metros de altura y III—Sujetos procedentes del nivel del mar y recién llegados a Morococha Las curvas han sido construidas con los datos correspondiente a la curva de disociación del O_2 en estos tres grupos

el nativo residente en la altura se debe a la diferente posición de la curva de disociación del O_2 y a su mayor contenido en la sangre arterial En un sujeto recién llegado a Morococha (4 540 metros) sin policitemia o con un grado muy moderado y sin cambios en la afinidad de la hemoglobina por oxígeno la cantidad de este gas liberado de la sangre a una presión de 25 mm Hg es muy inferior A pesar de constituir la policitemia un importante mecanismo adaptativo a la anoxia no creemos sin embargo que tenga desde este punto de vista una *importancia fundamental* o decisiva A juzgar por investigaciones que hemos llevado a cabo en los últimos años [40] parece que los mecanismos adaptativos de tal carácter residen a nivel de los tejidos y están relacionados con un grado de vascularización que permite una *mejor difusión del oxígeno* o con cambios químicos respiratorios en la utilización de este gas o con ambos procesos

6 *La policitemia del soroche crónico*

Monge [41] en 1928 indicó que la pérdida de la aclimatación a la altura que ocurre a veces en sujetos nativos o residentes permanentes está frecuentemente asociada a una acentuación anormal de la policitemia que corresponde al nivel de altura en que viven A esta condición se la ha denominado soroche crónico

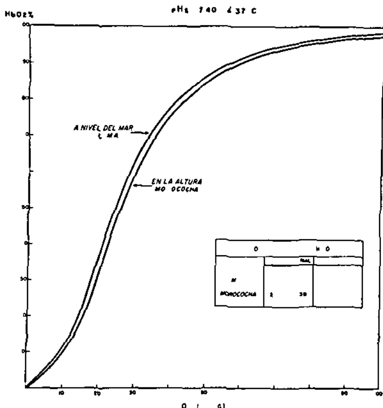


FIG 14 —Curva de disociacion del O_2 en sangre arterial a pH 7 40 y 37 C correspondiente a sujetos normales residentes en Lima al nivel del mar y en nativos residentes en Morococha a 4 540 metros de altura Tomada de Aste Salazar y Hurtado [38]

sangre arterial en virtud del aumento de la hemoglobina La difusion de este gas a los tejidos es favorecida también por la disminucion en la afinidad de la Hb por oxígeno característica que hemos constatado en los nativos residentes en la altura [38] y por el pH sanguíneo moderadamente disminuído aunque entre límites de variacion normal [39]

La curva de disociacion del oxígeno a un pH standard de 7 40 (Figura 14) está desviada a la derecha en los nativos residentes en Morococha a 4 540 metros (14 900 pies) de altura comparados con los sujetos normales residentes en Lima al nivel del mar [38] Este hallazgo ha sido confirmado recientemente con un mayor numero de observaciones que hemos realizado

Tomando en cuenta la posicion de la curva de disociacion del oxígeno y el contenido de este gas en la sangre arterial se puede calcular que el nativo residente y aclimatado a una altura de 4 540 metros libera 11 3 cc de oxígeno por cada 100 cc de sangre cuando esta encuentra una tension de O_2 de 25 mm Hg mientras que al nivel del mar *en el sujeto normal a esta misma presion* la sangre libera 10 2 cc o sea 11% menos (Figura 15) Esta condicion favorable en

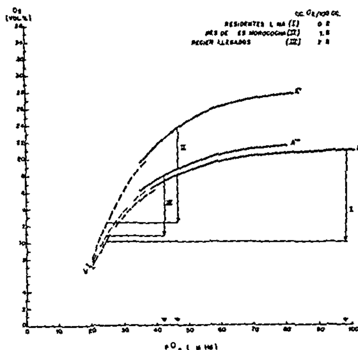


FIG. 15.—Cálculo teórico de la cantidad de O_2 liberada de la sangre a un pO_2 de 25 mm Hg en tres grupos de sujetos. I—Sujetos normales residentes en Lima al nivel del mar. II—Nativos residentes en Morococha a 4 540 metros de altura y III—Sujetos procedentes del nivel del mar y recién llegados a Morococha. Las curvas han sido construídas con los datos correspondientes a la curva de disociación del O_2 en estos tres grupos.

el nativo residente en la altura se debe a la diferente posición de la curva de disociación del O_2 y a su mayor contenido en la sangre arterial. En un sujeto recién llegado a Morococha (4 540 metros) sin policitemia o con un grado muy moderado y sin cambios en la afinidad de la hemoglobina por oxígeno, la cantidad de este gas liberado de la sangre a una presión de 25 mm Hg es muy inferior. A pesar de constituir la policitemia un importante mecanismo adaptativo a la anoxia no creemos, sin embargo, que tenga desde este punto de vista una importancia fundamental o decisiva. A juzgar por investigaciones que hemos llevado a cabo en los últimos años [40] parece que los mecanismos adaptativos de tal carácter residen a nivel de los tejidos y están relacionados con un grado de vascularización que permite una mejor difusión del oxígeno o con cambios químicos respiratorios en la utilización de este gas o con ambos procesos.

6. *La policitemia del soroche crónico*

Monge [41] en 1928 indicó que la pérdida de la aclimatación a la altura que ocurre a veces en sujetos nativos o residentes permanentes está frecuentemente asociada a una acentuación anormal de la policitemia que corresponde al nivel de altura en que viven. A esta condición se la ha denominado soroche crónico

eritremia de altura y enfermedad de Monge En los años transcurridos se han realizado diversas investigaciones sobre las características hematológicas de estos enfermos[42, 43] Todos ellos presentan un cuadro similar que puede ser ilustrado con la presentación de los datos referentes a un caso estudiado recientemente por nosotros

La saturación arterial al oxígeno está disminuída Los valores de hematíes hemoglobina y hematocrito están anormalmente elevados y hay un grado acentuado de reticulocitosis (Cuadro 5) Las plaquetas y leucocitos se encuentran normales La hiperbilirrubinemia es generalmente muy acentuada (sólo moderadamente en este caso) y es responsable de ella una elevación en la fracción indirecta Las protoporfirinas están altas Las cifras de volumen total de sangre circulante indican una intensa elevación de la masa globular y hemoglobina con disminución del volumen plasmático La excreción de urobilinógeno está aumentada con un alto índice hemolítico, característica esta última señalada originalmente por Merino[15] La eliminación de coproporfirinas no se encuentra por encima de los valores que corresponden a los nativos normales El examen de una biopsia medular reveló en este caso una marcadísima hiperplasia e hiperactividad del tejido eritroide y caracteres normales de las células mieloides Clínicamente estos casos están caracterizados por una cianosis intensa y un aspecto congestivo muy marcado

No es aún posible señalar con precisión el mecanismo patogénico del soroche

Cuadro 5 —*Valores hematológicos en un caso de Soroche Crónico estudiado en Morococha a 4 540 metros de altura comparados con los correspondientes a los nativos normales residentes a esta altura*

	Soroche crónico	Normal*
HbO_2 % Arterial	65 2	80 0
Hematíes (mill mc)	8 54	6 44
Hemoglobina (gr 100 cc)	25 33	20 13
Hematocrito (%)	79 0	59 5
Reticulocitos (mil mc)	239	46
Plaquetas (mil mc)	495	454
Leucocitos (mil mc)	8 70	7 04
Bilirrubina (mg 100 cc)	1 54	1 [illegible]
Protoporfirinas (µg %)	135	66
Volumen sangre (cc/kil)	122 9	103 7
Volumen plasma (cc/kil)	25 1	38 9
Volumen hematíes (cc/kil)	97 0	64 8
Cantidad de Hb (gr/kilo)	31 1	22 1
Excreción urobilinógeno (mg/día)	416	156
Indice hemolítico	26 1	13 5
Coproporfirinas (µg/día)	1 052	1 244

* En el nativo residente a una altura de 4 540 metros

crónico en lo que respecta a las alteraciones hematológicas a excepción de la abundante evidencia que existe para diferenciarlo en forma *definida* de la Policitemia Vera o enfermedad de Vaquez. Nosotros nos inclinamos a pensar que estas alteraciones son secundarias a un proceso pulmonar de fibro esclerosis posiblemente análogo al que corresponde a la enfermedad de Ayerza y que la acentuación anormal en el nivel de policitemia se debe al mayor grado de anoxemia presente en estos casos y que es consecuencia, a su vez de las alteraciones pulmonares. La reciente observación hecha por Rotta, Canepa y otros[44] en nuestros laboratorios de Morococha de una elevación moderadamente severa de la presión pulmonar (determinada por medio del cateterismo de las cavidades derechas del corazón y de la arteria pulmonar), en dos casos de soroche crónico auxilia a catalogar estos casos dentro del grupo de hipertensiones pulmonares.

No se puede sin embargo eliminar totalmente la posibilidad de que en algunos casos la acentuación anormal de la policitemia sea un fenómeno inicial y que se deba a la intervención de factores individuales no conocidos, que regulan la respuesta eritropoyética a un factor anóxico. Ya hemos indicado que en la altura hay un mayor grado de variabilidad en las características hematológicas. En estos casos las alteraciones pulmonares pueden ser efecto y no causa de la intensa policitemia que actúa sobre una red vascular con distensibilidad limitada a través de un gran aumento en la masa globular y viscosidad sanguínea. Es interesante notar la acentuación de los procesos de destrucción globular en el soroche crónico evidenciada por la elevada excreción de urobilinógeno y alto índice hemolítico. Parece tratarse de un esfuerzo de compensación a la mayor producción de hematíes y hemoglobina. Desde este punto de vista estos enfermos se encuentran dentro de un aparente círculo vicio o de formación y destrucción globular.

Finalmente es interesante señalar que tanto en estos casos de soroche crónico como en enfermos con Neumoconiosis con intensa policitemia a consecuencia de las alteraciones pulmonares y mayor grado de anoxemia, los valores más altos de hemoglobina en la sangre han sido siempre hallados entre 25 y 26 gramos por 100 cc, correspondiendo estas cifras al parecer a la máxima actividad eritropoyética encontrada en un medio elevado.

7. *La policitemia de altura en relación con otras policitemias secundarias y la Policitemia Vera*

La presencia de policitemia en un ambiente de baja presión y la relación que existe entre su nivel y el grado de anoxia sugiere que este factor es también el responsable de la frecuente elevación de hematíes y hemoglobina en procesos patológicos en los que existen dificultades para el suministro adecuado de oxígeno a los tejidos incluyendo el hematopoyético. Esto puede suceder cuando disminuye la adquisición de este gas por la sangre a nivel pulmonar, cuando hay alteraciones en su transporte por la hemoglobina circulante o en su distribución por la red vascular y finalmente cuando hay interferencia en su utilización por los tejidos. Es interesante señalar que dentro de la marcada variabilidad individual en el nivel de policitemia resultante en estas condiciones hay sin

embargo ciertas tendencias de orden general Una de ellas es la mayor intensidad de la respuesta eritropoyética en casos de fibro esclerosis pulmonar (Enfermedad de Ayerza) y en cardíacos congénitos y su frecuente ausencia en casos de enfisema pulmonar, a pesar de la anoxemia severa que generalmente se encuentra en estos enfermos La razon de esta discrepancia no es conocida pero puede estar relacionada con la intervencion de factores adicionales que condicionan o regulan la producción y nivel de la policitemia secundaria a la anoxia Es interesante a este respecto señalar que los cardíacos congénitos y los casos de fibro esclerosis pulmonar son los que mas se asemejan, en varios aspectos, a los sujetos aclimatados que viven en la altura y a quienes presentan síntomas de pérdida de esta aclimatación Tiene interés especial el estudio comparativo de la policitemia de altura tanto en sujetos normales como en casos de soroche cronico con la Policitemia Vera o enfermedad de Vaquez caracterizada tambien por un aumento absoluto e intenso de la masa globular circulante Para explicar la patogenia de esta ultima enfermedad se han invocado factores anoxicos,[45 47] como responsables de la hiperactividad hematopoyética Aparte de diferencias de orden clínico tal como la *ausencia de esplenomegalia en la policitemia* de altura existe otra que conceptuamos ser de caracter fundamental A juzgar por los datos que hemos presentado la anoxia por lo menos la originada por una menor tension de oxígeno en la sangre circulante constituye un estimulo restringido a la formacion de células eritroides y la resultante hiperactividad formativa de éstas se desenvuelve cuantitativamente aumentada pero cualitativamente normal Los leucocitos y plaquetas no son afectados Estas características *son siempre encontradas aun en casos de soroche cronico con intensa policitemia* En cambio es bien conocido el hecho de que en la Policitemia Vera se desarrollan, con frecuencia alteraciones en las celulas mieloides y megacariocitos y la maduración de los eritrocitos no siempre sigue un ritmo ordenado y fisiologico Estas diferencias hacen dudar de que la anoxia desempeñe un rol etiológico en esta enfermedad Además observaciones recientes [34 35] han demostrado la existencia de un contenido y saturación normal de oxígeno en el tejido medular en casos de Policitemia Vera

Resumen

La policitemia de altura presenta diferentes aspectos

1 *Influencia del grado y duracion de la anoxia* El grado de la policitemia tiene evidente relación con la duración y grado de la anoxia Cuando es de corta duración la respuesta policitémica puede faltar En cuanto al grado su efecto estimulante parece tener un límite pasado el cual se torna más bién en deprimente

2 *Policitemia en la aclimatacion natural a la altura* La policitemia de este tipo a una altura de 14 900 pies es de tipo absoluto con aumento del volumen de eritrocitos y disminución menos intensa del plasma y está asociada a una hiperactividad eritropoyética balanceada a su vez por un aumento proporcional de la destruccion globular Esta caracteristica explicaría la mayor tasa de eritroporfirinas hierro plasmático y el aumento en la eliminación intestinal y renal de pigmentos La elevacion de la bilirrubina plasmática de tipo indirecto tiene posiblemente igual explicación aunque puede estar también influída por una menor capacidad excretora del hígado consecuencia de la anoxia El estímulo del factor anóxico es específico de la eritropoiesis pues no aumenta la formacion de leucocitos y plaquetas y la respuesta eritropoyética se desenvuelve con un ritmo cualitativamente normal

3 *Desarrollo de la policitemia de altura* En las primeras horas de la llegada a la altura suele observarse aumento moderado de la Hb y de las proteínas del plasma a las 24–28 hs se inicia la reticulocitosis Determinando el volumen total de la sangre circulante se encuentra disminución del volumen plasmático Cuando la permanencia en la altura se prolonga se observa aumento progresivo del volumen total de eritrocitos estabilizándose la curva al octavo mes en un nivel más bajo que el de los nativos Las restantes alteraciones (hierro plasmático eritroporfirinas excreción de urobilinógeno) son en el mismo sentido que las observadas en los nativos

4 *Mecanismos de producción de la policitemia en la altura* En la policitemia inicial parecen intervenir dos mecanismos hemoconcentración y liberación de sangre almacenada especialmente en el bazo En la policitemia permanente se admite la acción estimulante eritropoyética de la menor tensión del oxígeno pero cabe preguntarse si esa acción estimulante se ejerce por vía humoral o directamente sobre el tejido hemopoyético inclinándose el autor hacia este último mecanismo

5 *La policitemia de la altura como mecanismo adaptativo* A pesar de ser la policitemia un importante mecanismo adaptativo a la anoxia los mecanismos fundamentales deben residir a nivel de los tejidos (grado de vascularización que permite mejor difusión del oxígeno cambios químicos respiratorios de la utilización de este gas o ambos procesos)

6 *Policitemia del soroche crónico o pérdida de la aclimatación a la altura de los nativos* Se manifiesta por una acentuación anormal de la policitemia y de todas las alteraciones antes señaladas Según el autor se debería a un proceso pulmonar de fibroesclerosis con la correspondiente exaltación de la anoxemia

7 *La policitemia de la altura en relación con otras policitemias secundarias y la policitemia vera* Aun cuando en las policitemias secundarias puede invocarse como factor causal la anoxia es curioso que la mayor respuesta eritropoyética se encuentra en la fibroesclerosis pulmonar y en los cardíacos congénitos mientras que está ausente en el enfisema pulmonar Hay pues que admitir factores adicionales que regulan la aparición y cuantía de las policitemias secundarias a la anoxia En cuanto a la policitemia vera parece muy dudoso que la anoxia desempeñe un papel etiológico

Altitude Polycythemia

It is an established fact that a low pressure atmosphere produces a polycythemic process in man The intensity of the polycythemia and its characteristics are influenced chiefly by the degree of anoxia and its duration which is the result of the decrease of the partial pressure of oxygen in the inspired air

Consequently altitude polycythemia is considered in the following aspects

(a) Polycythemia produced by temporary exposure to a low pressure atmosphere
Mechanisms of production and characteristics of the polycythemia

(b) Polycythemia in the natural acclimatization to altitude *Observations of native residents at an altitude of 4,000 meters (average barometric pressure 446 mm Hg)*
Relation to the degree of anoxemia
Role of polycythemia as adaptation mechanism
Variations in physical activity
Comparative study of polycythemia vera and other erythremic processes

(c) Polycythemia in acquired acclimatization *Observations of a group of normal subjects during a year's residence at an altitude of 4,000 meters*

(d) Polycythemia in the loss of natural or acquired acclimatization
Observations in cases of chronic Soroche

The data used for the discussion of altitude polycythemia include
Characteristics of the peripheral blood total volume of the circulating blood and its constituents
Hematopoietic activity bone marrow biopsy
Determinations of plasma iron protoporphyrines and coproporphyrines
Cell destruction plasma bilirubin and urobilinogen excretion in the urine and feces

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A-communication 1

Is Fibrinogen Utilized for Hematopoiesis in "Active" Polycythemia Vera? (With a Note on the Pathogenesis of the Hemorrhagic Manifestations in the Disease)

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Bleeding manifestations usually occurring sometime after minor trauma are observed during the course of "active" polycythemia vera. Analytical study of the hemostatic process in these patients reveals one outstanding abnormality, a low plasma fibrinogen level, all other factors of hemostasis being found normal and the platelets being not only elevated in number but also functionally normal. The deficiency of plasma fibrinogen ($1/3$-$1/4$ of normal) is even greater than shown by the chemical determinations when the reduction of the plasma volume in this disease as compared to the red cell volume is taken into consideration. Hence the clot is frail and tends to collapse, as few threads of fibrin are insufficient to retain the large number of red cells. One stage determinations of plasma prothrombin activity on undiluted plasma indicate hypoprothrombinemia. This is only apparent, however, and due to the effect of the low plasma fibrinogen concentration, since prothrombin activity of plasma becomes normal when the plasma is mixed 10% with fibrinogen-containing diluents. A similar situation is found in secondary polycythemia.

When the polycythemic process becomes "spent" and the red cell count and hemoglobin level fall (although white cell and platelet count may remain elevated), plasma fibrinogen level gradually rises to normal and the tendency to bleed subsides at the same time. If the red cell count and hemoglobin are reduced by treating patients of "active" polycythemia vera with myelosuppressive agents (TEM), the plasma fibrinogen level rises as red cell and reticulocyte count fall. When vice versa very active hematopoiesis and high reticulocytosis are induced by repeated venesections in patients with "active" polycythemia, plasma fibrinogen falls to very low levels.

In polycythemia vera then fibrinogen level of plasma varies in inverse proportion to the erythropoietic activity. Observations are being conducted to establish whether fibrinogen may be utilized for hematopoiesis in normal and pathologic conditions.

¿Es el Fibrinógeno Utilizado en la Hematopoyésis en la Policitemia Vera "Activa"? (Con una Nota Sobre la Patogénesis de las Manifestaciones Hemorragicas en esta Enfermedad)

Durante la policitemia vera activa se pueden observar después de un traumatismo leve manifestaciones hemorrágicas. Los estudios analíticos de los factores hemostáticos

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en estos enfermos revelan una marcada anormalidad un bajo nivel del fibrinógeno plasmático *en cambio los otros factores* de la hemostasia son normales las plaquetas se presentan en numero elevado y funcionalmente normales La deficiencia del fibrinógeno plasmático (1/3 a un 1/4 del normal) es aun mayor que la que se observa con las determinaciones comunes cuando se compara la reducción del volumen plasmático con la del volumen globular en esta enfermedad Por lo tanto el coágulo *es friable y tiende a romperse* porque las pocas fibras de fibrina son insuficientes para englobar al gran numero de glóbulos rojos La determinación de la actividad protrombínica (en un tiempo) en el plasma *no diluido* da como resultado hipoprotrombinemia Esta es sin embargo solo aparente y debida al efecto de la baja concentracion del fibrinógeno plasmático desde que se normaliza la actividad protrombínica del plasma cuando se mezcla el plasma con diluyentes que contienen un 10% de fibrinogeno

Cuando la policitemia se agota y el recuento de globulos rojos y nivel de hemoglobina disminuyen (a pesar de que la cifra de leucocitos y plaquetas puede mantenerse aumentada) el nivel del fibinógeno plasmático aumenta gradualmente hasta normalizarse al mismo tiempo que disminuye la tendencia a las hemorragias Cuando se reduce el numero de globulos rojos y hemoglobina en los enfermos con policitemia vera activa con agentes mielo upresivos (TEM) el nivel del fibrinógeno plasmático aumenta al mismo tiempo que disminuyen los globulos rojos y reticulocitos Cuando al contrario se incita una hematopoyesis muy activa y una alta reticulocitosis por medio de sangrías repetidas en enfermos con policitemia activa el nivel plasmático del fibrinógeno cae a niveles muy bajos

Pareceria por lo tanto que en la policitemia vera el nivel del fibrinógeno plasmático varia en relacion inversa a la actividad de la formacion de los globulos rojos Se investiga actualmente si la proteína es utilizada con fines hematopoyéticos

V communication 2

The Hematologic Picture of High Altitude

HUGO CHIODI*

The hematic pictures of permanent residents of Mina Aguilar province of Jujuy Argentina were studied at an altitude of 4500 and 4000 meters above sea level Polycythemia and increase of hemoglobin were observed the average values were in direct relation to the altitude at which the studied subject lived The globular values (mean volume mean hemoglobin and hemoglobin concentration) are normal from which is deduced that we deal with a polyglobulia of the normocytic type The sedimentation rate of the red cells increased granted the polyglobulia There is no relation between the length of permanence at that altitude or duration of the hypoxemia and the intensity of the hematic reaction The degree of relationship between the intensity of the hypoxemia and the hematic reaction in residents at the same altitude is established The average figures for leukocytes were found within normal limits With respect to the leukocyte formula an increase of the mononuclear elements and decrease of the neutrophile polynuclears was observed

The significance of the polyglobulia and increase of the hemoglobin as well as the mechanism of its production is discussed

El Cuadro Hemático de la Altura

Se estudio el cuadro hemático en residentes permanentes en Mina Aguilar Pcia de Jujuy Argentina a 4500 y 4000 m sobre el nivel del mar Se observo policitemia y au

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mento de la hemoglobina, estando los valores promedios en relación directa con la altura a que residían los sujetos estudiados. Los valores globulares: volumen medio, hemoglobina media y concentración de hemoglobina son normales, de lo cual resulta que se trata de una poliglobulia de tipo normocítico. La velocidad de sedimentación de los glóbulos rojos aumentó dada la poliglobulia. No existe correlación entre el tiempo de permanencia en la altura, esto es, duración de la hipoxemia y la intensidad de la reacción hemática. Se establece el grado de relación entre la intensidad de la hipoxemia y la de la reacción hemática de los habitantes a una misma altura. Las cifras promedios de leucocitos se hallaron dentro de los límites normales. En lo que respecta a la fórmula leucocitaria se observó un aumento de los mononucleares con disminución de los polinucleares neutrófilos. Se discute el mecanismo de producción y el significado de la poliglobulia y aumento de la hemoglobina.

V communication 3

Policitemias y Esplenectomía

LUIS DE MARVAL, ALBERTO S. SANTORO y JUAN R. GINESTA*

Motiva nuestro relato la observación de pacientes portadores de esplenomegalia, cuya etiología no se logró precisar y en quienes la exéresis del bazo configuró un síndrome clínico hematológico de policitemia.

Traemos para ello el estudio sucinto de tres de nuestros casos más típicos, cuyas historias serán publicadas in extenso, y que nos ha permitido ensayar una interpretación fisiopatológica que sometemos a consideración.

El primero, observado en el año 1935 por el Dr. Luis De Marval, se refiere a una mujer de 39 años de edad, en la que durante un examen obstétrico se le descubre una esplenomegalia. El embarazo terminó con un feto muerto. El estudio hematológico ulterior fué normal. Esplenectomizada, presenta tiempo después epistaxis, gingivorragias y hepatomegalia.

Dos años más tarde desarrolla clínicamente un síndrome policitémico con 8 millones de hematíes, 55 mil leucocitos y más de 2 millones de plaquetas. Ostealgias y hepatomegalia progresiva. *Las gingivorragias incoercibles y repetidas la llevan a la aplasia medular*, falleciendo en 1939.

El segundo pertenece al Dr. Juan R. Ginesta, se trata de una mujer que actualmente tiene 43 años de edad, que consulta en 1943 por cefaleas y polihipermenorrea, constatándosele una esplenomegalia. Hematológicamente fué normal. Fué esplenectomizada en noviembre del mismo año.

En 1945 tiene un embarazo que llega a término con parto normal. El niño tiene 7 años y no presenta estigma alguno que pudiera relacionarse con los antecedentes maternos.

Cuatro años después tiene una intensa hemorragia por una extracción dentaria. En Junio de 1950 epistaxis a repetición, ostealgias, hepatomegalia. Del estudio hematológico se deja sentado el diagnóstico de policitemia con [illegible] millones de hematíes, 40 mil leucocitos y una inusitada hiperplaquetosis.

Hace pocos meses tuvo una copiosa enterorragia que hizo que la cifra de hematíes *descendiera* a 5.210.000.

La tercera observación, corresponde al Dr. Alberto S. Santoro, es la de un hombre que actualmente tiene 55 años de edad. Presenta como antecedente remoto un ulcus duodenal a los 38 años.

En 1947 aqueja dolores intensos y progresivos en el hipocondrio izquierdo; la palpación comprueba un bazo grande. Previa punción esplénica y estudio hematológico que fueron normales, es esplenectomizado en Junio del mismo año.

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Al año de operado tiene una melena de regular importancia que posteriormente se ha repetido Gingivorragias espontáneas y una hemorragia por extracción dentaria

En 1960 es interpretado como un policitémico con el siguiente hemograma hematíes 7 millones leucocitos 40 mil e intensa hiperplaquetosis

Los tres pacientes presentan elementos semiologicos comunes y un curso evolutivo muy semejante que permiten hacer la siguiente sintesis esplenomegalias que son hallazgos casuales en dos de ellos sintomatología dolorosa en el otro no pueden ser ubicados en una entidad definida por falta de otros elementos patológicos

Esplenectomizados y transcurrido un tiempo variable presentan tendencia a las hemorragias En ese momento un nuevo estudio clínico hematológico descubre el síndrome policitémico con cifras celulares que varían para los hematíes entre 6 y 8 millones para los leucocitos entre 40 y 60 mil y para las plaquetas cifras superiores a 2 millones La crasis sanguínea es normal

Procurando una explicacion fisiopatológica de esta concatenación pensamos encontrarnos frente a policitémicos en potencia o latencia así consideremos a su médula osea normal o ya hiperplástica antes de la esplenectomia pero que liberada en su interrelación del freno esplénico vuelca al torrente circulatorio los elementos figurados expresión de su sobreactividad

Estímulos que no podemos precisar serían las causales de la esplenomegalia

El Profesor Dr José L Monserrat realizo el estudio histopatológico de los bazos de dos de nuestras enfermas y puede resumirse así ligera atrofia de los folículos blancos esclerosis de la capsula y tabiques aumento del retículo y de los elementos figurados de los cordones de Billroth En uno de ellos además se observan células gigantes mono y polinucleadas No nos permite pues hacer diagnóstico alguno ni presumir la evolucion que ulteriormente seguirán

Como los pacientes nos llegaron esplenectomizados y aceptando que las indicaciones podrían ser discutidas por no haber razones clínicas que las justificaran sacamos las siguientes conclusiones que las referimos en el orden de la aparicion de los hechos

1 Existirían policitemias con un período prolongado de latencia que tendrian en la esplenomegalia inespecífica la unica expresion clínica

2 La esplenectomía desencadena el síndrome policitémico

3 El bazo intervendría ostensiblemente en estos casos en la regulación de la mielopoyesis tal como en otras afecciones hematológicas

4 Estos enfermos presentan despues de la exéresis del bazo tendencia a las hemorragias pese a la extraordinaria trombocitemia que los puede llevar a la aplasia medular por agotamiento

5 Mejoran tratados como verdaderos policitémicos Uno se mantiene equilibrado con fósforo radioactivo el otro mediante substracciones de sangre

6 Finalmente diremos que frente a esplenomegalias aisladas sin reacciones mielohemáticas apreciables pasibles de un control especializado periodico no se justifica la esplenectomia pues la falta del freno como en estos casos puede dar lugar al síndrome que nos referimos

Polycythemias and Splenectomy

The authors conclude that

1 Polycythemias may occur preceded by a prolonged latent period during which the non specific splenomegaly can be the only clinical evidence of the disease

2 Splenectomy may set off in these cases the polycythemic syndrome

3 The role of the spleen in these cases would be the regulation of hemopoiesis such as happens in other hematologic entities

4 These patients show after splenectomy a bleeding tendency in spite of their elevated platelet counts which may take them on to an aplasia of the marrow due to the excessive demand on it

5 They improve when treated as true polycythemias One of our cases is kept under control with radio active phosphorous the other by bleeding therapy

6 Finally the authors maintain that when in the presence of isolated splenomegalies without appreciable hemopoietic disorders and with periodic medical control splenectomy is not justified because the lack of this inhibitor as seen in our cases may set off a true polycythemia

V-communication 1

Adaptaciones Hemodinamicas en las Anemias Tropicales

ALFREDO LANARI, AURELIO GARCIA SANTOS y COLON NUNEZ*

Se realiza el estudio hemodinámico de 11 enfermos con anemias tropicales de distinto grado de gravedad Se encuentra que la volemia se halla discretamente disminuída el volumen minuto circulatorio en reposo aumentado hasta tres veces los valores normales En ejercicio moderado el volumen minuto circulatorio alcanza en algunos hasta 6 veces los valores normales de reposo Hay una mejor correlacion con el descenso de la Hb aplicando el equivalente circulatorio que el índice cardíaco Por debajo de 5 gramos el volumen minuto circulatorio aumenta francamente

El análisis de la saturación de la Hb arterial demostro una neta insaturacion en los enfermos con menos de 6 gramos de Hb Se discute el por qué de este resultado paradojal considerando que tanto la desviacion de la curva hacia la derecha y la admisión veno a fisiológica de sangre muy insaturada pueden originar este aumento de la insaturacion arterial Respecto a la ventilacion en reposo y en el ejercicio existe un discreto aumento en ambas condiciones que se manifiesta aun cinco minutos después de terminado el ejercicio

Hemodynamic Adaptations in Tropical Anemias

A study of the hemodynamic conditions in 11 patients with tropical anemias of varying degree of severity is made It is observed that the volemia is slightly diminished the circulatory minute volume at rest augmented sometimes up to three times its normal value In moderate exercise the circulatory minute volume reaches in some cases up to six times its normal value at rest There is a better correlation between the decreased Hgb using the circulatory equivalent instead of the cardiac index Below 5 Gm of Hgb the circulating minute volume increases markedly

Analysis of the arterial Hgb saturation demonstrated a certain degree of unsaturation in the patients with less than 6 Gm of Hgb The reason for this paradoxical result is discussed considering that the deviation of the curve of Hgb to the right and the physiologic venous admission of very unsaturated blood might cause this increase of the arterial unsaturation Regarding ventilation at rest and in exercise a slight increase exists under both conditions which is noticeable even five minutes after the termination of exercise

Hospital Valenzuela Guayaquil Ecuador Trabajo realizado en la campana antituberculosa en cooperación entre el Gobierno del Ecuador y la Organizacion Mundial de la Salud

PART VI

Diagnosis of Hemolytic Disease, Treatment of Hemolytic Disease of the Newborn, Relationship of Immunology to Hematology

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Diagnóstico de la Enfermedad Hemolítica, Tratamiento de la Enfermedad Hemolítica del Recién Nacido, Relació entre Inmunología y la Hematología

Diagnóstico y Tratamiento de las Anemias Hemolíticas

CARLOS DA SILVA LACAZ*

LAS ANEMIAS hemolíticas constituyen enfermedades de gran interés clínico e inmuno-hematológico destacándose de sus distintos grupos la anemia hemolítica del recién nacido, afección para la cual el descubrimiento del factor Rh ha traído indiscutiblemente subsidios de gran valor en el esclarecimiento de su etiopatogenia. La extensión del asunto avaluada por la rica literatura a su respecto nos obliga en el presente relato al estudio del tema solamente bajo algunos aspectos, huyendo a discusiones de orden doctrinario encontradas en los compendios de hematología o de patología clínica.

Bases Generales para la Clasificación de las Anemias Hemolíticas. Diagnóstico Basado en el Estudio de Treinta y Siete Casos

Las anemias hemolíticas son comúnmente divididas en dos grupos fundamentales: las constitucionales o hereditarias y las adquiridas. Las primeras son debidas a un defecto intrínseco de los hematíes, siendo la anormalidad básica en la enfermedad, pues, de origen eritrocitario.

Las anemias hemolíticas adquiridas son debidas a factores extracorpusculares, pudiendo ocurrir sin causa aparente (idiopática o primaria) en el curso de enfermedades infecciosas, parasitarias o neoplásicas, o también como manifestación de hiperesplenismo traducido por eritrofagocitosis con inhibición medular.

Algunos casos de anemia hemolítica adquirida pueden ser explicados por mecanismo inmunológico activo, esto es, por la presencia en el suero de los pacientes de autoanticuerpos, algunos revelados solamente por medio de la prueba de Coombs o la de los hematíes tripsinizados. Autoanticuerpos para hematíes y plaquetas pueden ocurrir en un mismo suero, existiendo estrechas relaciones clínicas entre las anemias hemolíticas y la púrpura trombocitopénica primaria o idiopática.

Producidos los autoanticuerpos, éstos sensibilizarían los hematíes y así mismo las plaquetas. Poseyendo tales elementos avidez diversa para con las inmunoglobulinas, la crisis hemolítica o trombocitopénica estaría en dependencia de este factor, así como del pH del suero, temperatura, presencia de determinados iones y complemento. Todo nos hace creer que el bazo tiene participación en la elaboración de los autoanticuerpos, porque la esplenectomía en la mayoría de los casos de anemia hemolítica adquirida idiopática reduce considerablemente el título de los anticuerpos circulantes, lo que puede ser revelado por la prueba de Coombs indirecta y otros test serológicos.

En las anemias hemolíticas adquiridas sintomáticas o secundarias, muchas veces se producen estos anticuerpos, pero casi siempre en título bajo. En las anemias hemolíticas hereditarias o constitucionales (esferocitosis hereditaria

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siendo que diversos casos ya fueron registrados entre miembros de la misma familia. Casos de nacidos muertos en mujeres que presentaron crisis de hemoglobinuria nocturna paroxística fueron también relatados. Los datos de Butts (1945) no han sido confirmados. Para la caracterización de la hemoglobinuria paroxística recurrimos a la prueba de Ham cuyo principio es el siguiente: los hematíes de los enfermos son sensibles a sustancias líticas presentes en el plasma normal estando éste con pH ligeramente ácido (6 8 aproximadamente).

Un cierto número de datos inespecíficos para el diagnóstico diferencial de las *anemias hemolíticas orienta al clínico y al inmunohematólogo en el diagnóstico* sindrómico. Estos datos son principalmente: anemia, ictericia, hepatoesplenomegalia, reticulocitosis, excreción aumentada del urobilinógeno, esferocitosis y bilirrubina indirecta aumentada.

La demostración in vitro de una alteración de los hematíes para ciertas pruebas de hemolisis tales como la determinación de la resistencia globular, la prueba de la fragilidad mecánica, la prueba de la fragilidad de los hematíes a la incubación (autohemolisis) y el test de Ham (hemolisis en suero acidificado) deberán ser practicadas al lado de la verificación de autoanticuerpos aglutinantes.

Con cierta experiencia adquirida en los últimos años pensamos que la prueba de Coombs cuando es bien interpretada con los datos clínicos y hematológicos es de valor real en la diferenciación de los tipos hereditario y adquirido de las anemias hemolíticas. Es posible por pruebas de absorción según demostraron Crawford y Mollison (1951), la obtención de sueros antiglobulina capaces de reaccionar solamente con determinados tipos de hematíes sensibilizados posibilitando la preparación de reactivos dotados de mayor especificidad.

La prueba de los hematíes tripsinizados parece ser más sensible pero menos específica que el test de Coombs indirecto, pero está sujeta a una serie de errores que necesitan ser conocidos por el serologista. La cualidad de la tripsina y el período de tripsinización de los hematíes constituyen dos factores importantes que interfieren en el resultado de la prueba. La tripsinización en demasía determina *aglutinación de los hematíes en presencia de sueros normales, razón por la cual* debemos realizar el test de Morton y Pickles (1947) con pruebas de control. El test de los hematíes tripsinizados puede manifestarse positivo en sueros con hiperglobulinemia intensa (por γ globulina) como en el mieloma múltiple y en casos de lupus eritematoso difuso o sistemático. En las anemias falciformes ovalocíticas y del Mediterráneo la pruebe de Coombs resulta generalmente negativa.

Los pacientes con anemia hemolítica adquirida idiopática generalmente no toleran las transfusiones, indicando algunos autores el empleo de cortisona o de gasmo taza, los cuales reducirían el tenor de los autoanticuerpos preparándolos para el acto operatorio. Es difícil la valoración de los resultados obtenidos con los medicamentos en la desensibilización de estos pacientes. En algunos casos empero obsérvase reducción en el tenor de los anticuerpos. Los enfermos con anemia hemolítica adquirida idiopática con autoanticuerpo pueden sensibilizarse a distintos aglutinógenos sanguíneos ya que reciben generalmente transfusiones múltiples para el tratamiento de la afección de que son portadores.

y otras) la prueba de Coombs y la pesquiza de autoanticuerpos en medio salino y albuminoide casi siempre es negativa Nuestras observaciones concuerdan con las de la mayoría de los autores, demostrando la ausencia de mecanismo inmunologico para explicar la hemolisis que ocurre en tales casos En pacientes portadores de anemias hemolíticas, varias pruebas serologicas pueden ser ejecutadas, y sus resultados deben ser interpretados en union con los datos clinicos, anamnesticos, examen hematologico y mielograma Siguiendo la orientacion de Dacie (1950), hemos buscado verificar en pacientes de anemias hemolíticas la presencia de autoanticuerpos (libres o circulantes y fijos a los hematíes) por pruebas diversas, como ser test de Donath Landsteiner (criohemolisinas), busqueda de hemolisinas a treinta y siete grados de autohemoaglutininas a 2-5 grados diecisiete grados y treinta y siete grados y de anticuerpos incompletos Las pruebas de Coombs directa e indirecta, el test de los hematíes tripsinizados y la prueba de la elucion fueron por nosotros ejecutadas, como así mismo los examenes que revelan exceso en la formacion y excrecion de productos del metabolismo de la hemoglobina (bilirrubina plasmatica, urobilinogeno fecal, hemosiderinuria, etc) De acuerdo con un criterio inmunológico podemos considerar las siguientes afecciones capaces de provocar crisis hemoliticas

I *Mecanismo inmunologico ausente*
- (A) Anemias hemoliticas constitucionales
  - 1 Esferocitosis hereditaria o constitucional (tipo Minkowsky Chauffard)
  - 2 Anemia hemolítica familiar o hereditaria no esferocítica
  - 3 Anemia falciforme
  - 4 Anemia ovalocítica
  - 5 Anemia del Mediterráneo
- (B) Anemias hemoliticas adquiridas
  - 6 Anemias hemolíticas por hiperesplenismo
  - 7 Anemias hemolíticas por factores químicos (sulfanilamidas y derivados etc)

II *Mecanismo inmunologico presente*
  - 8 La anemia hemolítica adquirida idiopática
  - 9 Anemia hemolitica del recien nacido
  - 10 Anemia hemolítica postransfusional
  - 11 Hemoglobinuria paroxistica al frío (criohemoaglutininas)

III *Mecanismo inmunologico ocasionalmente presente*
  - 12 Anemia hemolítica adquirida y purpura trombocitopénica primaria
  - 13 Anemias hemolíticas sintomaticas o secundarias (enfermedad de Hodgkin y otras retículo endoteliosis estreptococias malaria cirrosis hepáticas leucemia neoplasias envenenamiento ofídico linfomas mononucleosis hepatitis infecciosa sarcoide de Boeck enfermedad de Gaucher neumonía atípica por virus y otras virosis principalmente la enfermedad de Newcastle lupus eritematoso difuso pénfigo foliáceo etc)

Butts en 1945 ha sugerido la posibilidad de que la hemoglobinuria nocturna paroxística sea debida a un fenomeno de sensibilizacion por el factor Rh a substancias existentes en el parasito de la malaria Los argumentos teoricos favorables a la hipotesis de Butts (1945) son los de que la hemoglobinuria paroxística es mas frecuente entre los blancos rara entre los negros y nativo

En dos casos de anemia hemolítica adquirida idiopática con prueba de Coombs negativa obtuvimos positividad de la prueba de los hematíes tripsinizados. En uno de esos casos la cortisona determinó franca mejoría clínica y hematológica con reducción de los anticuerpos incompletos revelados por el test de Morton y Pickles (1947).

En un caso de anemia hemolítica por accidente ofídico (serpiente no identificada) verificamos positividad de las pruebas de Coombs directa e indirecta, al paso que en otra observación de la misma naturaleza las referidas pruebas resultaron negativas.

In vitro, trabajando con venenos de *Bothrops cotiara*, *B. alternata*, *B. jararaca*, *Crotalus terrificus terrificus*, *B. atrox*, *B. jararacussu*, *B. bilineata* y *Lachesis muta* no hemos verificado sensibilización de los hematíes a tales zootoxinas capaz de ser revelada por la prueba de Coombs. *In vivo*, la positividad de la prueba de Coombs podría estar relacionada con diversos factores, inclusive con la liberación de principios activos de origen endógeno presentes en el plasma y que constituyen el mediador último de las funciones farmacológicas de aquellos venenos.

Tampoco con los venenos de *Lachesis muta*, de *B. jararaca* y de *B. jararacussu*, dotados *in vitro* de intensa actividad hemoaglutinante, hemos verificado la sensibilización de los hematíes humanos para el efecto aglutinante del suero antiglobulina. Como la tripsina y otras enzimas son capaces de sensibilizar los *hematíes para el efecto aglutinante de sueros bloqueadores anti Rh diluidos en* solución salina isotónica, hallándose dotados los venenos ofídicos de acción proteolítica, buscamos verificar si los mismos no sensibilizarían eritrocitos humanos para el efecto de anticuerpos incompletos Rh diluidos en solución fisiológica. Los resultados obtenidos fueron negativos.

En un caso de anemia hemolítica en el curso de neumonía atípica por virus observamos que la prueba de Coombs era positiva. En las demás observaciones la pesquiza de anticuerpos incompletos ha sido negativa, alejándose la posibilidad de un mecanismo inmunológico capaz de explicar la reacción hemolítica. La orientación terapéutica seguida en los casos de anemia hemolítica depende del *diagnóstico etiológico. Las transfusiones de sangre deberán ser practicadas en los* casos indicados, tomándose todos los cuidados en las pruebas cruzadas, principalmente en pacientes con anemia hemolítica adquirida idiopática. Con el empleo de la cortisona en pacientes con anemia hemolítica adquirida, la tolerancia a las transfusiones parece producirse de modo más satisfactorio con real *aprovechamiento de la sangre inyectada*.

La esplenectomía, principalmente en los casos con esplenomegalia acentuada, es la medida terapéutica indicada, obteniéndose resultados clínicos favorables cuando se le agregan transfusiones de sangre. Los hematíes del bazo en casos de anemia hemolítica idiopática son más fuertemente sensibilizados que los hematíes circulantes, *demostrando e en la pulpa esplénica de estos pacientes la presencia* de la prueba de Coombs. Fuera de este factor, la eritroestasis que se evidencia en aquel órgano aumenta la fragilidad de los hematíes al trauma y a las soluciones salinas hipotónicas.

En tales casos necesitamos diferenciar los autoanticuerpos de aquellos que se forman por transfusiones, generalmente los autoanticuerpos en medio salino y albuminoide, presentan títulos mas elevados cuando estan en presencia de los hematíes del paciente, al contrario de los anticuerpos formados después del empleo de transfusiones multiples El estudio de la sobrevivencia de los hematíes normales transfundidos a pacientes con anemia hemolitica presenta gran interes practico, verificandose que en los procesos hemoliticos hereditarios o constitucionales de origen no inmunologico los hematíes transfundidos sobreviven 120 días término medio al contrario de lo que sucede en las anemias hemoliticas adquiridas con anticuerpos circulantes y en las cuales las sobrevivencia es mas corta En 37 casos de anemias hemoliticas observados en el Hospital de Clinicas de San Pablo, la conducta que seguimos ha sido la siguiente

Examen clínico con rayos X de los huesos del craneo
Hemograma completo,
Determinacion de la resistencia globular,
Dosaje de las bilirrubinas
Pruebas de Coombs directa e indirecta,
Pesquiza de autohemoaglutininas en medio salino y en medio albuminoide,
Pesquiza de críohemoaglutininas
Otros examenes mielograma urobilinogeno fecal prueba de la dilucion test de los hematies tripsinizados reaccion de Paul Bunnell

Siguiendo esta orientacion fue posible clasificar los 37 casos de anemia hemolítica en los siguientes grupos

		Casos
1	Esferocitosis constitucional	13
2	Anemia hemolítica adquirida idiopática	6
3	Anemia hemolítica en el curso de la anemia falciforme	6
4	Anemia hemolítica por sulfamido derivados y otros agentes quimicos	2
5	Anemia hemolítica en el curso de la neumonía atípica por virus	1
6	Anemia hemolitica en el curso de la enfermedad de Hodgkin	1
7	Anemia hemolítica por mordedura de serpiente	2
8	Anemia hemolítica en el curso de enfermedad infecciosa febril	1
9	Anemia hemolitica en el curso de la leucemia linfática cronica	2
10	Anemia hemolítica familiar no esferocítica	1
11	Anemia de Cooley	1
12	Anemia hemolítica en el curso de la hemoglobinuria nocturna paroxistica	1

De 13 casos de anemia hemolítica constitucional solamente en uno la prueba de Coombs ha dado resultado positivo

En 6 observaciones de anemia hemolitica adquirida idiopatica la prueba de Coombs ha sido positiva en cuatro recomendandose siempre la practica simultanea de los tests directo e indirecto porque en ciertos casos con tenor reducido de anticuerpos incompletos la prueba indirecta puede resultar negativa al contrario del test directo En este caso los hematíes absorben en su superficie los anticuerpos circulantes pudiendo resultar la prueba directa positiva y la indirecta negativa La pesquiza de autohemoaglutininas en medio albuminoide mostrose superior a la misma prueba en medio salino

hepatoesplenomegalia Los casos graves de anemia hemolítica con tenor elevado de anticuerpos maternos y con prueba de Coombs positiva revelan hemolisis *intensa y rapida bilirrubina plasmatica elevada y el examen clinico muestra* señales evidentes de enfermedad hemolítica

Tales casos fueron tratados con exsanguinotransfusion por los Drs Oswaldo Mellone y Oscar Yahn

De mayo de *1949* hasta mayo de *1952* *36* casos de *enfermedad hemolitica* (forma de ictericia grave) fueron observados por nosotros y las principales conclusiones pueden ser resumidas así

I Numero de observaciones 36

a) Incompatibilidad al factor Rh 34 casos

b) Incompatibilidad al factor A 2 casos

En uno de estos casos la gestante era Rh negativa pero la sensibilización fué determinada por el factor A con prueba de Coombs directa negativa

Anticuerpos bloqueadores anti A fueron demostrados en el suero materno practicándose la absorcion de las aglutininas con substancia grupo-específica de Witebsky

II Causas de sensibilizacion materna

a) Sensibilizacion después de una gestacion 6 casos

b) Sensibilización por multiparidad 18 casos

c) Sensibilización por gestaciones multiples aliadas al empleo de la sangre Rh positiva 10 casos

d) Sensibilizacion de primigestas por transfusiones 2 casos

En doce casos verificamos pues sensibilizacion por transfusiones sanguíneas debiéndose *llamar la atencion una vez más sobre el relevante papel que los transfusionistas deben* desarrollar en la profilaxis de la anemia hemolitica practicando rutinariamente en los bancos de sangre la determinacion del factor Rh

III Datos hematologicos principales

A) Anemia observada en los 36 casos oscilando los glóbulos rojos entre 1 060 000 y 4 600 000 por mm

B) Hemoglobina osciló de 4 grs a 15 6 g %

C) Eritroblastos (numero para cada cien leucocitos)

	Casos
De 0 a 4 eritroblastos	8
De 4 a 10 eritroblastos	4
De 10 a 100 eritroblastos	13
De 100 a 200 eritroblastos	3
De 200 a 300 eritroblastos	3
De 300 a 400 eritroblastos	5

En ½ de los casos la eritroblastemia era pues prácticamente inexistente o discreta

IV Bilirrubinas (Dosadas en 2? casos por el método de Malloy Evelyn modificado por Ducci y Watson)

Bilirrubinemia indirecta

	Casos
0 a 10 mg %	5
10 a 20 mg %	9
20 a 30 mg %	10
30 a 40 mg %	3

Hubo elevacion de la bilirrubina indirecta en todos los casos con excepción de uno sometido al tratamiento sin los resultados de los exámenes por el antecedente de pérdida de un hijo tratado por la exsanguino transfusión Hubo elevacion simultánea de la bilirrubina directa en ?0 casos (mínimo de 0 5 mg % hasta un máximo de 30 5 mg %)

Anemia Hemolítica del recien nacido

En la ciudad de S Pablo, con la colaboracion de Oswaldo Mellone Oscar Yahn y Michel Jamra, hemos estudiado la anemia hemolitica del recién nacido sistematizando nuestras pesquizas de acuerdo con la siguiente orientacion

1 Verificacion de los anticuerpos Rh en gestantes Rh negativas trabajando con cuatro tecnicas a) *pesquiza de aglutininas en medio salino* b) *pesquiza* de anticuerpos bloqueadoses en medio albuminoide (albumina equina a 20 gramos %) c) pesquiza de anticuerpos reveladores por medio del test de Coombs y d) pesquiza de anticuerpos incompletos con hematíes tripsinizados La albumina equina que hemos preparado con la colaboracion de Ferri ha sido por nosotros igualmente utilizada en la preparacion de sueros bloqueadores anti Rh con buenos resultados en cuanto a la investigacion de anticuerpos incompletos por el test de Coombs indirecto nuestra experiencia confirma los datos obtenidos por otros autores mostrando sus ventajas sobre las pruebas practicadas en *medio albuminoide El test de Morton y Pickles (1947) parece ser mas sensible* que el de Coombs en la revelacion de anticuerpos incompletos pero debera ser practicado con controles para que los resultados obtenidos puedan ser debidamente comparados El período de tripsinización de los hematíes y el grado de pureza o de actividad de la tripsina constituyen algunos de los multiples factores capaces de alterar el resultado de la prueba

2 Determinacion del factor Rh del tipo sanguíneo del recién nacido así como la investigacion de la titulacion e identificacion de los anticuerpos Rh de origen materno en la sangre del cordon umbilical

3 Prueba de Coombs directa (sangre del cordon umbilical) En la preparacion del suero anti globulina preferimos siempre el metodo de Dalla Volta y Del Carpio (cocto antígeno) Para nosotros este procedimiento es superior al de Proom (1946) Los animales deberan ser sangrados definitivamente cuando el tenor de precipitinas alcanza 1 10 000 o 1 20 000 La liofilizacion del suero es una medida aconsejada En la imposibilidad de la liofilizacion podemos adicionar al suero de Coombs la azida de sodio o el Nipagin (ester metilico del acido oxibenzoico) al 1 1 000

4 Hemograma y dosaje de bilirrubinas en la sangre del recién nacido Trabajando en equipo nos ha sido posible reunir un pequeno grupo de casos bien estudiados del punto de vista clinico e inmunohematologico De acuerdo con los resultados de las pruebas inmunohematologicas y del examen clinico orientamos el tratamiento transfusional Dejamos de consignar los casos tratados con transfusion de sangre total o de globulos Rh negativo con el fin de considerar solamente las observaciones en las cuales fué practicada la exsanguinotransfusion La transfusion de sangre total o de globulos Rh negativo ha quedado reservada para casos benignos de anemia hemolítica con bajo tenor de anticuerpos en la sangre materna y prueba de Coombs negativa o ligeramente positiva y tardía revelando el hemograma anemia ligera siendo igualmente ausente o de pequena *intensidad la ictericia observada Casi siempre el* examen clinico revela estado general satisfactorio del recien nacido estando ausente la

tininas a 2-5 a 1 y a 3 por medio de las pruebas de Coombs directa e indirecta de los hematíes tripsinizados y de la elucion para la investigación de anticuerpos incompletos Asimismo determinaciones de bilirrubina plasmática de urobilinógeno fecal de hemo i derinuria

En base a tales estudios las anemias hemolíticas pueden clasificarse en I) *Mecanismo inmunológico ausente* A) anemias hemolíticas constitucionales (esferocitosis hereditarias anemia hemolítica familiar no esferocítica anemia falciforme anemia ovalocítica anemia del Mediterráneo) B) anemias hemolíticas adquiridas (por hiperesplenismo por factores químicos) II) *Mecanismo inmunológico presente* (anemia hemolítica adquirida idiopática anemia hemolítica del recién nacido anemia hemolítica post transfusional hemoglobinuria paroxística a frígore) III) *Mecanismo inmunológico ocasionalmente presente* (anemia hemolítica adquirida y purpura trombocitopénica primaria anemias hemolíticas secundarias)

Analizando las distintas pruebas el autor se refiere a la utilidad del test de Ham (hemolisis en suero acidificado) en la hemoglobinuria paroxística nocturna a la resistencia globular osmótica mecánica y al calor Destaca el valor de la prueba de Coombs sobre todo para la diferenciacion de los tipos adquirido y congénito La prueba de la tripsina más sensible que la anterior es menos específica y sujeta a mayores posibilidades de error (positiva en hipergammaglobulinemias) Señala también la utilidad de la determinacion de la sobrevida de los hematíes

Es de notar la frecuente intolerancia para las transfusiones de estos enfermos atenuable por el empleo de cortisona y gas mo taza debida a la facilidad de sensibilizacion a diversos aglutinógenos sanguíneos siendo necesaria la diferenciación entre los autoanticuerpos existentes y los anticuerpos creados por las transfusiones

Siguiendo las orientaciones antedichas ha sido posible clasificar los 3 casos observados por el autor en el Hospital de Clínicas de San Pablo de la siguiente manera 1) esferocitosis constitucional 13 casos en uno solo de los cuales fue positiva la prueba de Coombs 2) anemia hemolítica adquirida idiopática 6 casos (en 4 Coombs positiva y en los 2 restantes tripsina positiva) 3) en el curso de anemia falciforme 6 casos todos con Coombs negativa 4) por sulfoderivados y otros agentes químicos 2 casos con Coombs negativa 5) en el curso de neumonitis a virus 1 caso con Coombs positiva 6) en el curso del Hodgkin 1 caso Coombs negativa 7) por mordedura de serpiente 2 casos uno de ellos con Coombs positiva en contradiccion con las experiencias realizadas in vitro en tal sentido 8) en el curso de enfermedad infecciosa febril (1 caso) 9) en leucemia linfática cronica (2 casos) 10) an hemol fam no esferocitica (1 caso) 11) anemia de Cooley (1 caso) y 12) hemoglobinuria paroxística nocturna (1 caso) todas estas ultimas con prueba de Coombs negativa

Referente al tratamiento el autor destaca el valor de las transfusiones de sangre siempre que se tomen las debidas precauciones de la cortisona (en especial para el mejor aprovechamiento de aquéllas en las formas adquiridas) y de la esplenectomia principalmente en los casos con esplenomegalia acentuada

Anemia hemolítica del recién nacido Fueron realizadas las siguientes pruebas 1) Investigacion de anticuerpos anti Rh en gestantes rh negativas mediante a) busqueda de aglutininas en medios salino b) de anticuerpos bloqueadores en medio albuminoso (albumina equina al 20%) c) prueba de Coombs y d) de la tripsina Ha sido de gran valor el test de Coombs 2) Determinacion del factor Rh del grupo sanguineo del recién nacido identificacion y titulacion de anticuerpos anti Rh maternos en la sangre del cordon 3) Prueba de Coombs directa en la sangre del cordon 4) Hemograma y bilirrubinemia en el recién nacido Para la preparacion del suero antiglobulinico prefiere el autor la técnica de Dalla Volta y Del Carpio utilizando la azida de sodio o el Nipagin para su conservación

En 56 casos observados desde 1949 hasta 1952 en colaboracion con los doctores Mellone Nahn y Jamka el autor llega a las siguientes conclusiones A) producidos por incompatibilidad al factor Rh 34 casos al factor A ? casos estos ultimos con Coombs negativa (se demuestra la presencia de anticuerpos bloqueadores anti A en el suero materno luego le

V *Frecuencia de la enfermedad hemolítica por los diversos aglutinógenos*

De los 36 casos de anemia hemolítica del recién nacido con prueba de Coombs positiva hemos podido caracterizar el anticuerpo Rh en 21 eventualidades obteniéndose los siguientes resultados

	Casos
Anticuerpos Rh_0 (D)	6
Anticuerpos Rh_1 (CD)	13
Anticuerpos Rh_2 (DE)	*1*
Anticuerpos Rh_1 Rh_2 (CDE)	1

Resultados obtenidos Evolucion

En los 12 primeros casos fue empleada la técnica de Wiener En los demas hemos pasado a emplear la técnica de la vena umbilical, posible en 20 casos y abandonada en 4 por la imposibilidad de identificacion de la vena En la mayoria de los casos fue empleada sangre total Rh negativa en el volumen de un litro

En algunos casos empleamos sangre preservada de 24 a 48 horas haciendose la extraccion de por lo menos 100 cm³ de plasma sobrenadante con el fin de concentrar la sangre introducida De 36 casos 7 obitos ocurrieron atribuidos a

a) Tratamiento tardio 4 casos
b) Muerte por toxicosis con fenomenos dispépticos 2 casos
c) Muerte por posible tromboflebitis de la vena cava después del cateterismo de la vena umbilical 1 caso

Los examenes neurologicos practicados hasta el momento en los 29 casos revelan en uno secuelas neurologicas muy evidentes (a los 20 meses no habla, no anda y presenta incordinacion motora) y en otro secuelas discretas (pequeno retardamiento en el sector motor)

En 31 casos ha sido posible hacer examenes repetidos de los hematíes después *del tratamiento mostrando el hemograma lo siguiente*

a) Elevacion de los globulos rojos en 28 casos
b) Elevación de la hemoglobina en 27 casos
c) Caída acentuada del numero de eritroblastos en 30 casos

La bilirrubina fue repetida en 15 casos observándose caída de la bilirrubina *indirecta en todos ellos Se observo asimismo elevacion de la bilirrubina directa* total en nueve casos (posible alteracion hepatica) Caída de la bilirrubina total en seis casos

Resumen

Las anemias hemolíticas pueden ser dividas en 2 grandes grupos 1) las constitucionales o hereditarias por un defecto intrínseco del hematíe y 2) las adquiridas idiopáticas o secundarias de causa extracorpuscular En muchas de las ultimas en especial las idiopáticas puede descubrirse un mecanismo inmunológico (presencia de auto anticuerpos revelables por las pruebas de Coombs o de la tripsina) Dichos auto anticuerpos actuan sensibilizando los hematíes y en determinados casos a las plaquetas asignándose al *faro* un importante papel en su produccion Las secundarias suelen tener un título de anticuerpos más bajo En las formas constitucionales y hereditarias salvo rara excepcion falta el mecanismo inmunologico

Para el estudio se han realizado las siguientes determinaciones aparte del examen clínico y hematologico demostracion de autoanticuerpos (libres o fijos a los hematíes) busqueda de hemolisinas frías (Donath Landsteiner) hemolisinas a 37 autohemoaglu

of virus pneumonitis 1 case with positive Coombs (6) in Hodgkins disease one case with negative Coombs (7) due to snake bite 2 cases (one of them with a positive Coombs contradicting in vitro experiments) (8) during the course of febrile infectious disease one case (9) in chronic lymphatic leukemia 2 cases (10) non-spherocytic familial hemolytic anemia 1 case (11) Cooley's anemia 1 case (12) nocturnal paroxysmal hemoglobinuria 1 case (all with negative Coombs)

With reference to the treatment the author points out the value of *blood transfusions* when the necessary precautions are taken cortisone (especially for a better transfusion therapy in the acquired form) and splenectomy principally in the cases with marked *splenomegaly*

Hemolytic Anemia of the Newborn

The following tests were made (1) *Investigation of anti Rh antibodies in pregnant* Rh negative women by means of (a) search for agglutinins in saline media (b) blocking antibodies in albumin (bovine albumin 20%) (c) Coombs test and (d) trypsin test The Coombs test has been of great value (2) Determination of Rh factor and blood group of the newborn and identification and titration of maternal anti Rh antibodies in the cord blood (3) Direct Coombs test on cord blood (4) Blood counts and bilirubinemia in the newborn For preparing anti globulin serum the author prefers the technique of Dalla Volta and Del Carpio utilizing sodium azide or Nipagin for its conservation In 36 cases seen since 1949 to 1952 together with Doctors Mollone Yahn and Jamca the author reaches the following conclusions (a) due to Rh incompatibility 34 cases group A incompatibility 2 cases these with negative Coombs test (the presence of anti A blocking antibodies in maternal serum is shown after the absorption of agglutinins with Witebsky's specific substance) (b) *sensitization in primagravidas due to transfusions* 2 cases (in all the rest of the cases *sensitization followed two or more pregnancies with* in 10 of these a history besides of Rh positive transfusions) (c) anemia from 1 060 000 to 4 600 000 hemoglobin between 4 and 15 6 gm % erythroblastemia practically non present in one third of the cases (d) indirect serum bilirubin elevated in all cases except for one (e) in 21 cases the type of anti Rh antibody was identified the most frequent being the anti Rh (CD) followed by the anti Rh (D) anti Rh (DE) and the anti Rh Rh (CDE)

In respect to treatment except in the mild cases the exchange transfusion was preferred the site of choice being the umbilical vein Rh negative whole blood was generally used fresh or relatively fresh and in some occasions packed in an approximate volume *of one litre* Results *seven deaths in 36 cases treated and neurological* sequelae in two cases severe in one and mild in the other

absorber las aglutininas con substancia grupo específica de Witebsky B) sensibilización en primigestas por transfusiones 2 casos en todos los demás la sensibilizacion fué con secutiva a dos o más embarazos (en 10 de estos ultimos casos existía además el antecedente de transfusion Rh positiva C) anemia entre 1 060 000 y 4 600 000 Hb entre 4 y 15 6 g % eritroblastemia prácticamente inexistente en ⅓ de los casos D) elevacion de la bilirrubina indirecta en todos los casos menos uno E) en 21 casos se caracterizo el tipo de anticuerpo anti Rh causal siendo el más frecuente el anti Rh_1 (CD) y luego el anti Rh (D) el anti Rh_2 (DE) y el anti Rh_1Rh_2 (CDL)

Acerca del tratamiento salvo los casos leves se ha preferido el uso de la exsanguíneo transfusión con preferencia por la vena umbilical Se utilizó generalmente sangre total rh negativa fresca o poco estacionada en ocasiones concentrada en volumen aproximado de 1 litro Resultados 7 muertes en 36 casos tratados y en 2 casos secuelas neurologicas graves en 1 y leves en el restante

Diagnosis and Treatment of the Hemolytic Anemias

The hemolytic anemias can be divided into two large groups (1) the constitutional or inherited group due to an intrinsic defect of the red cell and (2) the acquired secondary or idiopathic anemias arising from extracellular factors In many of the latter especially the idiopathic group *an immunologic mechanism can be determined* that is the presence of auto antibodies which can be detected by the Coombs or a trypsine test These auto anti bodies act by sensitizing the red cell and in some cases also the platelets The spleen appears to have an important role in its production The secondary anemias usually have a lower titer of antibodies In the constitutional or inherited group except in rare instances this immunologic mechanism is not found

For studying these anemias various tests were used apart from the clinical and hema tologic investigations These were the direct and indirect Coombs test for detecting auto antibodies (free or fixed to the red cell) cold agglutinins hemolysins at 37 C and auto hemoagglutinins at 2 5 17 and 37 C *Incomplete antibodies* were *investigated by* the use of trypsinized red cells and by elution procedures Serum bilirubin fecal uro bilinogen and hemosiderinuria were also determined

On the basis of such studies the hemolytic anemias can be classified as to (1) *absent immunological mechanism*—(a) constitutional or congenital hemolytic anemias (inherited spherocytosis non spherocytic familiar hemolytic anemia sickle cell anemia ovalocytic anemia and mediterranean anemia)—(b) acquired hemolytic anemias (due to hypersplen ism or chemical factors) (2) *immunological mechanism present* (acquired idiopathic hemo lytic anemia hemolytic anemia of the newborn post transfusional hemolytic anemia paroxysmal hemoglobinuria a frigore) (3) *immunological mechanism occasionally present* (acquired hemolytic anemia and primary thrombocytopenic purpura secondary hemolytic anemia)

Analyzing these different tests the author underlines the usefulness of the Ham test (hemolysis in acidified serum when studying nocturnal paroxysmal hemoglobinuria) the fragility test (osmotic mechanical and heat) and mainly the Coombs test most valu able for differentiating the acquired and congenital types The trypsin test more sensitive than the above is less specific and subject to wider misinterpretations (positive in hyper globulinemias) The author also shows the utility of cell survival determination

These patients show a frequent intolerance to *transfusions (which can be attenuated* by the use of cortisone and nitrogen mustard) due to a tendency towards sensitization to different blood antigens making necessary the differentiation between the existing auto antibodies and the ones originated by previous transfusions

Following the procedures outlined it has been possible to classify 37 cases as follows (1) constitutional spherocytosis 13 cases (only one of whom showed a positive Coombs test) (2) acquired idiopathic hemolytic anemia 6 cases (four had positive Coombs and the other two positive tryp in tests) (3) sickle cell anemia 6 cases (all Coombs negative) (4) due to sulfa drugs and other chemicals 2 cases with negative Coombs (5) in the course

TABLE 1 — Anti Rh serum (Bellas) vs Rh_1 Cells

Serum dilution	Saline	Serum
Undil	++++	++++
1 2	++++	++++
1 4	++++	++++
1 8	++++	++
1 16	+++	++
1 32	++	+
1 64	+	+
1 128	+	+
1 256	−	−
0	−	−

− = no agglutination ± = faint agglutination + = slight agglutination ++ = marked agglutination +++ = strong agglutination ++++ = very strong agglutination

TABLE 2 — Anti Rh Serum (Bur) vs Rh Pos Cells

Serum dilut	Saline	Serum
Undil	−	++++
1 2	−	++++
1 4	−	++++
1 8	−	++++
1 16	−	++++
1 32	−	++++
1 64	−	+++
1 128	−	+++
1 256	−	+++
1 512	−	++
1 1024	−	+
0	−	−

immunization in pregnancy appeared to be on shaky ground for a while Only after the significance of the diluents used in Rh agglutination was recognized mainly as a result of investigations by Diamond[3] and Wiener[4] was considerable experimental evidence in favor of Levine s theory accumulated

Tables 1 and 2 illustrate this development The first table records the titration of the serum of an Rh negative mother who had given birth to an erythroblastotic baby * Her serum contained an Rh antibody of fairly high titer demonstrable even when saline solution was used as a diluent as did Levine and Stetson s first case The serum of the second Rh negative mother (Bur) would have failed to reveal the presence of Rh antibodies if saline solution had been used exclusively as a diluent However potent Rh antibodies were indeed present in the serum as easily detected when normal serum was used as a diluent

In this and the following experiments decreasing amounts of the patient s serum (volume 0 1 cc) were each mixed with 0 1 cc of cell suspension (about 2%) After being kept for 1 hour at 37 C they were centrifuged slightly and read for agglutination

VI 2

The Immunology of Acquired Hemolytic Anemia Diagnostic and Therapeutic Considerations

ERNEST WITEBSKY*

ACQUIRED hemolytic anemia may be divided both from the serological and clinical points of view into two main groups (1) the disease in the newborn and (2) the disease as it occurs in adults In both instances the patient's cells are sensitized presumably by antibodies directed against the patient s own blood cells The work of Coombs Mourant and Race[1] has given a new tool to the investigator allowing the easy recognition of sensitization in both clinical entities Indeed striking similarities in the serological characteristics of both diseases have induced observers to consider them as caused by the same mechanism the Coombs test being the common denominator Yet, closer scrutiny reveals important differences justifying a restatement of facts

I *Hemolytic Disease of the Newborn*

Incompatibility of the major blood groups was considered by several investigators particularly Hirszfeld many years ago as a possible cause of certain complications in pregnancy Thanks to Levine and his co workers the pathogenesis of the hemolytic disease of the newborn is today clearly understood and the concept of isoimmunization in pregnancy established beyond any doubt *We are now well aware that other factors beside the Rh factor may* cause isoimmunization of the mother and result in the birth of an erythroblastotic child But whatever factor may be the cause of isoimmunization in pregnancy, the main principle *in all instances is the presence of an antigenic factor in the baby s* blood cells which is absent in the mother The leakage of the baby s blood cells into the mother s circulation elicits the production of isoantibodies in the mother s body Sensitization never takes place if the mother s cells contain the same factor as the baby s cells and we recognize in this basic principle the operation of Ehrlich s concept of horror autotoxicus the fear of self poisoning In other words autoantibodies are never produced and do not come into play in the pathogenesis of erythroblastosis

In retro pect the ingenuity of Levine and Stetson[2] was probably favored by the Goddess of Good Luck when they observed their first case of isoimmunization in pregnancy because this first case exhibited an isoagglutinin in the mother s blood serum demonstrable with the old fashioned serological technic using saline solution as a diluent In subsequent cases however such an antibody was not found again for quite some time As a matter of fact the entire theory of iso

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TABLE 5.—*Titration of Cord Serum (Bur.) against Rh Positive Cells*

Serum dilutions	Saline	Serum	Serum albumin
Undil	–	–	+++
1:2	–	–	++++
1:4	–	–	++++
1:8	–	–	+++
1:16	–	–	+++
1:32	–	–	++
1:64	–	–	+
1:128	–	–	+
1:256	–	–	–
1:512	–	–	–
1:1024	–	–	–
0	–	–	–

efforts to connect Rh antibody functions with the clinical picture of erythroblastosis in the baby. Such an example is illustrated in table 4. The superiority of the serum albumin mixture as a diluent over both saline and plain serum is very evident in this anti-Rh serum. In all of our experiments the diluent mentioned (saline, serum or serum albumin) was always used in the preparation of both the serum and the cell suspensions.

Many cord sera of erythroblastotic babies which failed to show the presence of circulating Rh antibodies when saline or even serum was used as a diluent now clearly demonstrated the presence of Rh antibodies with serum albumin mixture as the diluent, as shown in table 5. Without the use of serum albumin as a diluent Rh antibodies of considerable strength would have been overlooked.

In all instances described so far, normal adult serum was used as a diluent. Actually, the capacity of normal serum to enhance Rh agglutination develops during ontogenesis and depends upon the age of the fetus (see table 6). The cord sera of fetuses of different ages are here compared. The umbilical cord serum of the 6 month old premature fetus practically lacked the enhancing

TABLE 6.—*Comparison of Human Adult Serum and Cord Serum on Activation of Incomplete Rh Antibodies*

Strength of anti-Rh serum	Saline	Adult serum	Umbilical cord sera			
			6 mo	8 mo	2 wk premat	Full term
1:5	+	++++	±	++	+++	+++
1:10	–	++++	–	+	+++	+++
1:20	–	+++	–	–	+	+++
1:40	–	+++	–	–	±	+
1:80	–	++	–	–	–	±
1:160	–	+	–	–	–	–
0	–	–	–	–	–	–

The cell suspensions as well as serum dilutions were prepared in the respective diluents mentioned, i.e., the cord serum of 6 mo. fetus, 8 mo. fetus, full term baby, etc.

TABLE 3 — *Agglutination of Rh Positive Group O Cells by the Serum of Mrs Kle and the Cord Serum of Baby Kle*

Serum dilution	Mother's serum		Cord serum	
	Saline	Serum	Saline	Serum
Undiluted	+	++++	–	+++
1:2	+	++++	–	+++
1:4	±	++++	–	+++
1:8	–	++++	–	+++
1:16	–	++++	–	+++
1:32	–	++++	–	++
1:64	–	++++	–	+
1:128	–	+++	–	+
1:256	–	+++	–	±
1:512	–	+++	–	–
1:1024	–	++	–	–
0	–	–	–	–

Obviously the demonstration of free circulating Rh antibodies in an erythroblastotic baby's own body is of great importance both from the understanding of the pathogenesis of the disease and for diagnostic purposes. Table 3 compares the Rh antibody titer of a sensitized Rh negative mother with that of the cord serum of her baby. In this case again the mother's Rh antibody is demonstrable in high titer only when serum is used as a diluent. The same holds true for the baby's cord serum, although the Rh antibody titer here is characteristically lower than in the mother's serum.

There still remained a considerable percentage of cases of erythroblastotic babies whose Rh negative mothers' serum apparently were free of Rh antibodies even when normal serum was used as a diluent. However, when albumin or better still (at least in our hands) a mixture of equal amounts of 30% bovine albumin and serum was used, Rh antibodies of considerable titer could be demonstrated in most of the remaining cases which heretofore had resisted the

TABLE 4 —*Anti Rh Serum (Woloridge) vs Rh_0 Cells*

Serum dilution	Saline	Serum	$\overline{AA}$ serum albumin
Undil	++	+++	++++
1:2	+	++	++++
1:4	+	++	++++
1:8	–	+	++++
1:16	–	+	++++
1:32	–	+	++++
1:64	–	+	++++
1:128	–	–	+++
1:256	–	–	++
1:512	–	–	+
1:1024	–	–	+
0	–	–	–

TABLE 5.—*Titration of Cord Serum (Bur.) against Rh Positive Cells*

Serum dilution	Saline	Serum	Serum albumin
Undil	–	–	+++
1 2	–	–	++++
1 4	–	–	++++
1 8	–	–	+++
1 16	–	–	+++
1 32	–	–	++
1 64	–	–	+
1 128	–	–	+
1 256	–	–	–
1 512	–	–	–
1 1024	–	–	–
0	–	–	–

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Strength of Rh serum	Saline	Adult serum	Umbilical cord			
			6 mo	8 mo	2 wk premat	Full term
1 5	+	++++	±	++	+++	+++
1 10	–	++++	–	+	+++	+++
1 20	–	+++	–	–	+	+++
1 40	–	+++	–	–	±	+
1 80	–	++	–	–	–	±
1 160	–	+	–	–	–	–
0	–	–	–	–	–	–

The cell suspensions as well as serum dilutions were prepared in the respective diluents mentioned, i.e., the cord serum of 6 mo fetus, 8 mo fetus, full term baby, etc.

TABLE 7.—*Development of Activating Capacity of Sera of Normal Newborns on Incomplete Rh Antibodies*

Diluted anti-Rh serum	Saline	Adult serum	Sera of full term newborns					
			Baby Va			Baby Ha		
			1st day	2nd day	3rd day	1st day	2nd day	3rd day
1:40	−	+++	−	++	++	−	−	−
1:80	−	++	−	+	+	−	−	−
1:160	−	++	−	±	±	−	−	−
1:320	−	+	−	−	−	−	−	−
0	−	−	−	−	−	−	−	−

properties and behaved almost like saline solution.[5] Some enhancing properties appeared in the cord serum of the other two premature babies, while the full term baby contained the properties to a greater extent though not equal to normal adult serum as first noticed by Wiener.[6] Quantitative differences between cord sera of full term babies and adult sera can better be recognized if rather diluted Rh antisera of relatively weak potency are used.

The development of the enhancing properties after birth of full term babies is shown in table 7. Blood specimens from two babies were taken on the first, second and third day after birth. As can be seen, one of the two babies developed some enhancing properties on the second day after birth, while the other baby was still free of enhancing properties even on the third day of life and might not show it for several months according to Gurevitch and associates.[7]

It is obvious, therefore, that the diluent used is of greatest importance in the demonstration of Rh antibodies and other isoantibodies. Race uses the terms "complete" and "incomplete" to differentiate between antibodies demonstrable in saline solution and in a protein diluent. For practical purposes we can differentiate at present three main types of diluents, i.e., (1) saline solution, (2) normal adult serum, (3) a serum albumin mixture. The simultaneous use of these three diluents reveals marked differences in isoantibody functions, and it is our belief that there actually exist different varieties of Rh antibodies.[8] There still remains a small percentage of isoantibodies, Rh antibodies as well as others (especially the Duffy type of antibody),[9] which fail to agglutinate sensitized cells even in a serum albumin mixture. The sensitization of blood cells by this type of isoantibodies *can be shown so far only by the Coombs technic.* We are looking forward, therefore, to the discovery of a fourth type of diluent, so to speak, which would bring about visible agglutination of cells sensitized by this last variety of antibody.

It is possible to separate the various types of Rh antibodies, to a certain extent at least, by dialysis. This method points to the existence of qualitative rather than quantitative differences of Rh antibodies. Hill and Haberman,[10] using another method, also have been able to separate the different varieties of Rh antibodies. Sturgeon and Brown[11] failed to obtain similar results by means of electrophoresis using as test cells the unique and highly unusual D positive cells described by Race, Sanger and Selwyn.[12]

TABLE 8 — *Agglutination of A_1 Cells by Immune Anti-A Serum**

Serum dilution	Not neutralized			Neutralized†		
	Saline	Serum	Ser. alb.	Saline	Serum	Ser. alb.
1:2	++++	++++	++++	–	+++	+++
1:4	++++	++++	++++	–	+++	+++
1:8	++++	++++	++++	–	+++	++
1:16	++++	++++	++++	–	++	+
1:32	++++	++++	++++	–	++	–
1:64	++++	++++	++++	–	+	–
1:128	+++	++++	+++	–	+	–
1:256	++	++++	+++	–	–	–
1:512	+	++++	++	–	–	–
1:1024	–	+++	+	–	–	–
1:2048	–	++	–	–	–	–
0	–	–	–	–	–	–

* Patient delivered a slightly erythroblastotic baby (Sol.)
† Serum neutralized by *addition of 1 part A and B Substances (Sharp & Dohme chemical vial)* to 1 part patient's serum

The importance of the type of diluent selected in the study of isoagglutination caused by *antibodies other than Rh antibodies is equally impressive*. As an example, the serum of a woman was selected containing an isoagglutinin anti-A_1 of the immune type. This woman, belonging to blood Group O, had given birth to a baby suffering from a mild case of erythroblastosis. The titration of this serum against A_1 cells using the different diluents is illustrated in table 8. As *can be seen, normal adult serum* when used as a diluent reveals a higher titer of A_1 antibodies than does saline solution. The serum albumin mixture, on the other hand, is definitely inferior to undiluted adult serum though slightly better than saline solution. Following neutralization of this serum with the isolated group specific substance A, anti-A antibodies no longer can be recognized when saline is used as a diluent. Using normal serum as a diluent, anti-A antibodies of a lower titer are still detected.[19] The enhancing influence of normal serum as a diluent in the demonstration of immune anti-A_1 antibodies, and its relative superiority to serum albumin mixture is fairly characteristic for immune anti-A_1 and anti-B *antibodies, though not necessarily a requisite*.

It is by no means possible, therefore, to predict which diluent might be the most suitable for the detection of certain isoagglutinins. For instance, an interesting picture is obtained if saline is compared with serum as a diluent in titrating even *normal human serum against blood cells of subgroups* A_1 and A_2, as shown in table 9. The normal serum of Group B agglutinates cells of the subgroup A_1 somewhat better when serum instead of saline solution is used as a diluent. Such behavior is not infrequently seen even in the absence of any known immunization of the patient under investigation. However, A_2 cells are agglutinated considerably stronger *in saline solution than in normal serum*, an observation which seems to be a fairly constant characteristic of the A_2 agglutination. The inferiority of serum as a diluent in the agglutination of A_2 cells should be remembered in the daily practice of blood group determination.

TABLE 9 — *Agglutination of A_1 and A_2 Cells by Normal Human Group B Serum*

Normal gr B serum	Saline		Serum	
	A_1	A_2	A_1	A_2
Undiluted	++++	+++	++++	+++
1 2	++++	+++	++++	++
1 4	+++	+++	++++	+
1 8	+++	++	++++	−
1 16	+++	+	++++	−
1 32	+++	+	++++	−
1 64	++	−	+++	−
1 128	++	−	++	−
1 256	−	−	+	−
1 512	−	−	−	−
1 1024	−	−	−	−
0	−	−	−	−

The Detection of Sensitization of Baby's Blood Cells in Erythroblastosis

From the diagnostic point of view the direct Coombs test has proven an invaluable tool for detecting sensitization of the newborn baby's blood cells. The addition of antiglobulin antisera to the thoroughly washed baby's cells results in prompt agglutination. However the same baby's cells if suspended in normal adult serum without being touched by saline solution show spontaneous agglutination especially if the test is carried out on the slide.[14] Sometimes we add cell sediments by means of an applicator directly into the drop of normal serum and suspend the cells within the drop by stirring. It should be reemphasized that sensitized cells prepared for the purpose of the Coombs test by thorough repeated washings often fail to show agglutination when suspended in normal adult serum. We have made use of this slide agglutination test for diagnostic purposes for years in our laboratory and it is my understanding that several other laboratories use the same procedure. In our laboratory we first referred to this test as the "Nine Drop Test" because of the nine different mixtures used for routine purposes at that time as recorded in table 10. The red blood cells of the severely erythroblastotic baby are strongly agglutinated in normal adult serum in contrast to the normal Rh positive and Rh negative cells used as controls. At the same time an anti Rh (anti D) serum agglutinates both the baby's cells and the normal Rh positive cells. No agglutination occurs in saline solution.

TABLE 10 — *"Nine Drop Test" for Detection of Sensitization of Cells of Baby Kl**

Diluent	Cells		
	B by Kl	Norm Rh pos	Normal Rh neg
Undil human adult serum	++++	−	−
Anti Rh serum incomplete	++++	++++	−
Saline (control)	−	−	−

* Severely erythroblastotic baby

TABLE 11 —*Slide Agglutination Test in Erythroblastosis due to Rh Sensitization*

Diluent	Rh positive control cells	Baby's cells
Serum + albumin	–	++++
Normal adult serum	–	–
Anti D serum	++++	++++
Saline sol	–	–

Severely erythroblastotic

Table 11 illustrates a further step in the study of the phenomenon of spontaneous agglutination of sensitized baby's cells In this case the cord blood cells of the severely erythroblastotic baby were strongly agglutinated upon suspension in the serum albumin mixture whereas the normal serum diluent failed to bring about agglutination There is not always such a marked difference between serum albumin and normal adult serum Usually however serum albumin proves to be the superior diluent for detecting sensitization of the erythroblastotic baby's cells This is not surprising considering that the Rh antibodies in the mother's blood (which actually permeate the placenta and sensitize the baby's cells) are also better demonstrable in serum albumin mixture than in plain serum As a matter of fact the addition of albumin to the erythroblastotic baby's cells suspended in its own cord serum will frequently produce satisfactory agglutination

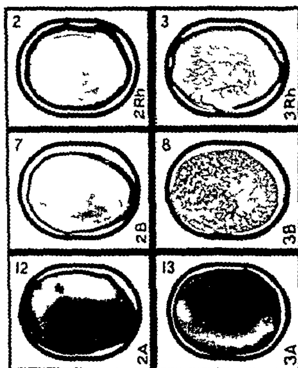


FIG 1

TABLE 9 — *Agglutination of A_1 and A_2 Cells by Normal Human Group B Serum*

Normal gr B serum	Saline		Serum	
	A_1	A_2	A_1	A_2
Undiluted	++++	+++	++++	+++
1:2	++++	+++	++++	++
1:4	+++	+++	++++	+
1:8	+++	++	++++	−
1:16	+++	+	++++	−
1:32	+++	+	++++	−
1:64	++	−	+++	−
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1:512	−	−	−	−
1:1024	−	−	−	−
0	−	−	−	−

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TABLE 10 — *"Nine Drop Test" for Detection of Sensitization of Cells of Baby Kle*

Diluent	Cells		
	Baby Kle	Normal Rh pos	Normal Rh neg
Undil. human adult serum	++++	−	−
Anti-Rh serum, incomplete	++++	++++	−
Saline (control)	−	−	−

Severely erythroblastotic baby

TABLE II.—*Slide Agglutination Test in Erythroblastosis due to Rh Sensitization*

Diluent	Rh pos. control cells	Baby's cells
Serum + albumin	−	++++
Norm. adult serum	−	−
Anti D serum	++++	++++
Saline sol.	−	−

Severely erythroblastotic

Table II illustrates a further step in the study of the phenomenon of spontaneous agglutination of sensitized baby's cells. In this case the cord blood cells of the severely erythroblastotic baby were strongly agglutinated upon suspension in the serum albumin mixture, whereas the normal serum diluent failed to bring about agglutination. There is not always such a marked difference between serum albumin and normal adult serum. Usually, however, serum albumin proves to be the superior diluent for detecting sensitization of the erythroblastotic baby's cells. This is not surprising, considering that the Rh antibodies in the mother's blood (which actually permeate the placenta and sensitize the baby's cells) are also better demonstrable in serum albumin mixture than in plain serum. As a matter of fact, the addition of albumin to the erythroblastotic baby's cells suspended in its own cord serum will frequently produce satisfactory agglutination

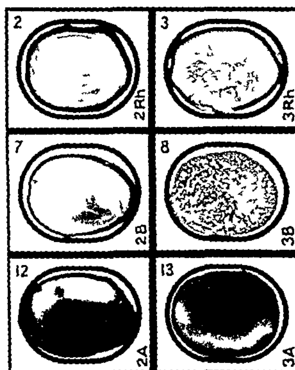


FIG. I

TABLE 12.—*Slide Agglutination Test in Erythroblastosis Caused by Sensitization against the A Factor*

	Normal gr A cells	Baby's (gr A) cells
Serum + albumin	—	+
Norm adult serum	—	++
Coombs serum	—	—
Saline sol	—	—

Cells tested by this method were washed 4 times

In our own laboratory we prefer to use both diluents, i e normal adult serum and serum albumin mixture because of the additional information we obtain regarding the type of Rh antibodies involved Figure 1 visualizes the actual appearance of the test, which is carried out in much the same manner as an Rh determination on the slide The picture illustrates only six drops—on the one side the control cells (2 7, 12) and on the other side the erythroblastotic baby's cells (3 8, 13) In the first row (2 and 3) normal adult serum was used as a diluent, in the second row (7 and 8) serum albumin mixture and in the third row (12 and 13) saline solution Only the baby's sensitized cells appear to be agglutinated and definitely stronger in serum albumin mixture (3 plus)

Cases of erythroblastosis caused by sensitization against the A factor exhibit a picture which differs in an interesting way from Rh cases as illustrated in table 12 The baby s cells which were sensitized by immune anti A_1 antibodies are better agglutinated in normal adult serum than in the serum albumin mixture in direct contrast to cells sensitized by the Rh antibody Agglutination in many cases of A B sensitization is weaker than in Rh sensitization but the superiority of normal serum over serum albumin mixture as a diluent seems to be characteristic of sensitization due to the A B factors We are often led to suspect this type of sensitization by the reverse action of the serum and serum albumin diluents (Again the behavior of the baby s cells sensitized by an immune anti A_1 antibody parallels the observation noted in titrating the immune anti A antibody in the mother s serum where serum as a diluent was superior to a serum albumin mixture) The Coombs test is frequently negative or questionable Recent reports of van Loghem [15] of Dacie [16] and of Crawford and Mollison [17] on the presence of multiple antibodies in antiglobulin sera might help to shed a light on the seemingly negative Coombs test in sensitization due to the blood group factors A and B The immune anti A antibody might be connected with a different fraction of serum globulin than the Rh antibody and it seems therefore that the observations of these investigators might hold promise for a better understanding of the mechanism of the Coombs test as it applies to the detection of blood cell sensitization caused by different types of antibodies

Treatment

Inasmuch as normal adult serum and plasma seem to enhance agglutination of sensitized baby s cells to a considerable extent one might doubt the wisdom of transfusion of whole blood if one is permitted to compare experiences obtained

in vitro with those in vivo For transfusion purposes, it would seem to be preferable to remove the plasma from the blood and inject the blood cells only (or washed cells) instead of whole blood Whole blood transfusions might not be objectionable however in cases where Rh antibodies are enhanced by serum albumin mixture only and not by serum or plasma alone One might speculate that erythroblastotic babies transfused successfully with whole blood might have been sensitized by an Rh antibody which was not enhanced by serum or plasma alone but only by higher concentrations of albumin as in the serum albumin mixture used as an experimental tool in the test tube only It is granted though that such an assumption might very easily overdo the correlating of laboratory findings and clinical reactions The exchange transfusion using whole blood undoubtedly is still the method preferred by the majority of investigators and correctly so, because in this way up to 90% of the sensitized baby s cells are removed and replaced by normal nonsensitized cells

II *Acquired Hemolytic Anemia of the Adult*

There is an amazing resemblance between the laboratory findings of many (though not all) cases of acquired hemolytic anemia of the adult and cases of hemolytic disease of the newborn Here too the direct Coombs test is positive Here too the patient s cells spontaneously agglutinate when mixed on a slide in normal adult serum or serum albumin as shown in table 13 The patient s cells spontaneously agglutinate markedly in normal adult serum as well as in serum albumin mixture The direct Coombs test carried out on the patient s cells is strongly positive However the patient s cells washed as for the Coombs test are no longer agglutinated in normal adult serum

The similarity in the serology between erythroblastosis of the newborn on the one hand and acquired hemolytic anemia of the adult on the other hand, goes even farther as shown in table 14 The cells of this patient suffering from severe acquired hemolytic anemia are strongly agglutinated only in a serum albumin mixture but fail to be agglutinated in plain adult serum Surprisingly the direct Coombs test repeated on several occasions remained negative which might be explained by the previously mentioned recent investigations of Crawford and Mollison and of van Loghem Usually the direct Coombs test runs parallel to the spontaneous slide agglutination test

TABLE 13 —*Tests for Acquired Hemolytic Anemia of Adult*

	Normal cells (Rh + gr A)	Patient's cells (Rh + gr A)
I Slide Sensitization Test (Unwashed cells)		
Normal adult serum (gr A)	–	++++
Serum albumin mixture	–	++++
II Coombs Test (Cells washed 3 times)		
Human serum antiserum (rabbit)	–	++++
Normal adult serum (gr A)	–	–

TABLE 12.—*Slide Agglutination Test in Erythroblastosis Caused by Sensitization against the A Factor*

	Normal gr A cells	Baby's (gr A) cells
Serum + albumin	–	+
Norm adult serum	–	++
Coombs serum*	–	–
Saline sol	–	–

* Cells tested by this method were washed 4 times

In our own laboratory we prefer to use both diluents, i e, normal adult serum and serum albumin mixture, because of the additional information we obtain regarding the type of Rh antibodies involved Figure 1 visualizes the actual appearance of the test which is carried out in much the same manner as an Rh determination on the slide The picture illustrates only six drops—on the one side the control cells (2 7 12) and on the other side the erythroblastotic baby's cells (3 8 13) In the first row (2 and 3) normal adult serum was used as a diluent, in the second row (7 and 8) serum albumin mixture and in the third row (12 and 13) saline solution Only the baby's sensitized cells appear to be agglutinated and definitely stronger in serum albumin mixture (3 plus)

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Inasmuch as normal adult serum and plasma seem to enhance agglutination of sensitized baby's cells to a considerable extent one might doubt the wisdom of transfusion of whole blood if one is permitted to compare experiences obtained

close similarity of the laboratory findings Hemolytic disease of the newborn is caused by isoimmunization the antibody being produced in a different individual i e the mother and *passively* transferred to the baby The pathogenesis of hemolytic disease of the newborn as due to isoimmunization seems to be proven beyond doubt In contrast acquired hemolytic anemia of the adult, as explained by an autoantibody would represent the sequela of *active* immunization against an antigen presumably present in the patient s own cells contradicting the otherwise generally operating law of horror autotoxicus The immunologist therefore should keep an open mind for the present at least toward the theory of autosensitization as the cause of acquired hemolytic anemia of the adult which would assume the presence of an autoantibody *active at body temperature* In the face of this dilemma it might be well to seek additional causes to explain the important laboratory findings of red blood cell sensitization

Certain viruses recently have been accused of being responsible for sensitization of the patient s cells and further studies of the virus etiology of acquired hemolytic anemia might be expected In the meantime the pathogenesis of acquired hemolytic anemia of the adult and the problem of the origin and nature of the sensitizing agent still remain challenging mysteries

Inmunologia de la anemia hemolitica adquirida consideraciones Diagnósticas y Terapeuticas

Existen dos grupos principales enfermedad en el recién nacido y en el adulto En ambas los hematíes del paciente se combinan con anticuerpos en su propia circulación Esta sensibilización puede ser reconocida *in vitro* por la prueba de Coombs Aunque parecen existir grandes semejanzas el examen inmunológico profundo revela grandes diferencias

I *Enfermedad hemolítica del recién nacido*

En todos los casos hay un factor de naturaleza antigénica en los hematíes del niño que está ausente en la madre y estimula en ésta la produccion de iso anticuerpos Sin embargo el concepto de Ehrlich sobre el horror autotoxicus se mantiene en todos los casos pues no se produce un anticuerpo contra un antígeno existente en el propio individuo

El suero normal de adultos permite la demostración de las aglutininas Rh cuando fracasan las soluciones salinas La albumina o todavía mejor la mezcla suero albumina es superior al suero Como diluyente el suero del cordón umbilical no tiene la capacidad reforzadora del suero adulto y el suero del feto de 6 a 7 meses casi carece de dicha propiedad

La sensibilizacion de los hematíes del recién nacido se demuestra fácilmente por la prueba directa de Coombs y también puede ser demostrada suspendiendo los hematíes sensibilizados en suero de adulto o mejor en suero albumina que provocan aglutinacion espontánea de los globulos sensibilizados

Tratamiento Es aconsejable la transfusion de glóbulos lavados para evitar el efecto reforzador del plasma La mayoría de los investigadores todavía prefieren la exanguino transfusión con sangre completa

II *Anemia adquirida del adulto*

Existe gran semejanza en los hallazgos de laboratorio entre muchos casos de anemia hemolitica del adulto y la enfermedad del recién nacido prueba directa de Coombs positiva aglutinación espontánea de los hematíes del paciente cuando se mezclan sobre un porta objeto con suero normal o mezcla de suero albumina Con frecuencia existen en esos pacientes inmuno iso anticuerpos que se confunden fácilmente con autoanticuerpos

Tratamiento Es preferible la transfusión de globulos en vez de sangre total La causa del posible efecto dañino del plasma requiere nuevos estudios y no puede ser comprendida

TABLE 14

Slide Sensitization Test of Patient Tasca—Gr O Rh_1 Homo (CDe/Ce)

Diluent	Tasca cells O		Normal cells			
			Rh + O		Rh – O	
Ser alb mixt	++	++++	–	–	–	–
Serum gr O	–	–	–	–	–	–
Saline	–	–	–	–	–	–

1st reading—after 10 minutes 2nd reading—after 15 minutes

Coombs Test of Patient Tasca

Diluent	Tasca cells O	Normal cell (Rh + O)
Anti human ser	–	–

We were interested in studying the possible difference between normal adult serum and the serum of patients with acquired hemolytic anemia as to their properties to enhance agglutination of sensitized cells We have the impression that in some instances during certain periods of the disease (especially in the early stages) these enhancing properties are weaker in the patient's serum than in normal serum It should be understood that serologic investigations carried out on the serum of patients suffering from acquired hemolytic anemia are fraught with difficulties These patients seem to have a tendency to develop all kinds of isoantibodies of known and unknown specificity which are easily mistaken for autoantibodies In addition the presence of cold agglutinins complicates the picture The only proper way to prove the presence of autoantibodies of course would be to use the patient s own cells as test cells However because the patient s cells are heavily sensitized they constitute a very poor tool for experimental purposes

Treatment

Observations of Dr Dameshek and other leading hematologists have already pointed out the possible damaging effect of normal plasma in the transfusion of patients suffering from acquired hemolytic anemia As a matter of fact we have the impression that the death of one of our own patients following several whole blood transfusions was due to the plasma part of the whole blood transfused Obviously therefore *cell* transfusions would seem preferable to *whole blood* transfusions in spite of the fact that so far experimental evidence of the damaging effect of normal adult plasma in cases of acquired hemolytic anemia of the adult is not so convincing as in cases of hemolytic disease of the newborn

The role of splenectomy and treatment with ACTH and cortisone is at the present time subject to analysis and review and should be discussed by our clinical colleagues

III *Conclusion*

There are fundamental differences between the two groups of acquired hemolytic anemia in regard to the *origin* of the sensitizing antibody in spite of the

close similarity of the laboratory findings Hemolytic disease of the newborn is caused by isoimmunization the antibody being produced in a different individual i e the mother and *passively* transferred to the baby The pathogenesis of hemolytic disease of the newborn as due to isoimmunization seems to be proven beyond doubt In contrast acquired hemolytic anemia of the adult as explained by an autoantibody would represent the sequela of *active immunization against* an antigen presumably present in the patient s own cells contradicting the otherwise generally operating law of horror autotoxicus The immunologist *therefore* should keep an open mind for the present at least toward the theory of autosensitization as the cause of acquired hemolytic anemia of the adult which would assume the presence of an autoantibody *active at body temperature* In the face of this dilemma it might be well to seek additional causes to explain the important laboratory findings of red blood cell sensitization

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INMUNOLOGIA DE LA ANEMIA HEMOLITICA ADQUIRIDA CONSIDERACIONES DIAGNOSTICAS Y TERAPEUTICAS

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Tratamiento Es preferible la transfusión de globulos en vez de sangre total La causa del posible efecto dañino del plasma requiere nuevos estudios y no puede ser comprendida

tan fácilmente como la enfermedad del recién nacido La esplenectomía y el tratamiento con ACTH y cortisona exigen también nuevos análisis

III *Conclusion*

A pesar de las semejanzas existen diferencias fudamentales en los dos grupos respecto al origen del anticuerpo sensibilizante La enfermedad del recién nacido es causada por iso inmunizacion siendo el anticuerpo producido en la madre y transferido al nino En cambio la enfermedad del adulto explicada por un anticuerpo representaría la secuela de una inmunizacion activa contra un antígeno presente en los propios globulos del paciente contradiciendo la ley del horror autotoxicus

Por ahora está justificado que el inmunólogo se ponga en guardia frente a esa teoria de la auto sensibilizacion que presupone la presencia de un auto anticuerpo activo a la temperatura del cuerpo Valdría la pena buscar causas adicionales que expliquen estos fenómenos Se está estudiando el papel de los virus que podría traer nuevos progresos en esta dirección

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VI 3

Blood Groups and Genetics

R R RACE*

IN 1900 one blood group system was known to exist the ABO system In discovering these differences in blood Landsteiner discovered a new and quite unsuspected branch of human physiology and the knowledge that has been gained in this field has contributed not only to medicine through blood transfusion and through hemolytic disease of the newborn but also it can be said to have laid a firm foundation for the study of human genetics It is also now contributing a great deal to anthropology

In the 52 following years eight more blood group systems have been discovered and have been worked out in detail they are shown in the first chart in the order of their discovery

These nine blood group systems together with the curious ability of some people to taste phenyl thio-carbamide while others cannot are the only normal physiological human characteristics of which the manner of inheritance is known with certainty Sex has not been put on the list because though we know the controlling part played by the X and Y chromosomes we know nothing yet of the genes involved

In man there are 24 pairs of chromosomes Ordered in a line along them are the genes the genes of everything we inherit Hundreds perhaps thousands of genes on each chromosome genes for health genes for happiness genes for musical ability and so on and so on Yet of all these thousands of genes which must exist the only healthy normal ones yet identified are those responsible for the 10 characters shown in the first chart

Many genes responsible for rare abnormalities have been identified and the place on the sex chromosomes of one or two of them even located Hemophilia for example is caused by a gene carried on the upper part of the larger of the 2 sex chromosomes the X chromosome and near it is the gene for red green color blindness

The manner of inheritance is known of such conditions as Huntington s chorea tuberose sclerosis albinism six fingers etc but all these abnormalities are much too rare to be any use as labels for the chromosomes carrying the particular gene The blood groups on the contrary are not rare but are to be found in

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tan fácilmente como la enfermedad del recién nacido La esplenectomía y el tratamiento con ACTH y cortisona exigen también nuevos análisis

III *Conclusion*

A pesar de las semejanzas existen diferencias fudamentales en los dos grupos respecto al origen del anticuerpo sensibilizante La enfermedad del recién nacido es causada por iso inmunización siendo el anticuerpo producido en la madre y transferido al nino En cambio la enfermedad del adulto explicada por un anticuerpo representaría la secuela de una inmunización activa contra un antígeno presente en los propios globulos del paciente contradiciendo la ley del horror autotoxicus

Por ahora está justificado que el inmunologo se ponga en guardia frente a esa teoría de la auto sensibilización que presupone la presencia de un auto anticuerpo activo a la temperatura del cuerpo Valdría la pena buscar causas adicionales que expliquen estos fenómenos Se está estudiando el papel de los virus que podría traer nuevos progresos en esta dirección

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the time will come when groupless blood substitutes alone will be transfused and when fetal immunization of the mother will be prevented as a routine Then to do a direct anti globulin test on the red cells of a newborn baby may seem as archaic as it now would to cast his horoscope But in the science of genetics blood groups or the compounds they represent will continue to be studied perhaps for centuries

The brilliant discovery by Levine and his colleagues that hemolytic disease of the newborn was due to a difference between the Rh groups of the mother and of her child was of course a major contribution to the still somewhat small volume of scientific medicine

One effect of Levine s discovery *has been to provide a great stimulus to the* teaching of elementary genetics to students of medicine Mothers of children who have had hemolytic disease are often surprisingly well read in the subject and a physician will probably make rather a poor show if his knowledge is less than the mother s if for example he does not appreciate *the significance in these* families of the Rh positive father being heterozygous rather than homozygous

I do not think there is anything new to be said of the serologic diagnosis of hemolytic disease If the red cells from the umbilical cord give a good positive direct anti globulin test then the child has hemolytic disease due to anti Rh or much more rarely to anti Kell

In the much more uncommon type of this disease due to anti A or anti B the direct anti globulin test is negative or only weakly positive A diagnosis of hemolytic disease due to anti A or anti B is difficult to establish I cannot speak from personal experience but the most convincing fact would seem to be the demonstration by cell survival counts that A cells or B cells as the case may be are destroyed if transfused into the baby while O cells given at the same time survive normally This test must seldom be practicable and usually an opinion has to be based on the combined results of various tests on the mother s serum the partial neutralization test of Witebsky for example or the shift in optimal temperature the hemolytic titer and the difference between the albumin titer and the saline titer

No important advances have been made in the methods of detecting anti Rh in maternal serum since 1947 when Morton and Pickles introduced us to their trypsin test Nevertheless the detection of anti Rh is becoming increasingly efficient owing to two causes First the albumin indirect anti human globulin *and trypsin tests are being more and more widely used and second* the practice of testing as a routine the blood of pregnant women is becoming more and more general In England all women who attend the state ante natal clinics have their Rh groups done and the serum of all those who are Rh negative and of those *who are Rh positive but who have a suggestive history* is screened for anti Rh either by the albumin or by the trypsin method

Chart 1 —*Ten Fixed Points on the Chromosomes of Man*

1 ABO blood groups A_1A_2 subdivisions	1900 1911
2 MN blood groups MNSs subdivisions	1927 1947
3 P blood groups	1927
4 Ability to taste phenyl thio carbamide	1931
5 Secretion of the ABO antigens in saliva Lewis blood groups closely associated	1932 1946
6 Rh blood groups Many subsequent subdivisions	1940
7 Lutheran blood groups	1945
8 Kell blood groups	1946
9 Duffy blood groups	1950
10 Kidd blood groups	1951

every family and they make ideal labels for the chromosomes carrying their genes

Somewhere on these chromosomes are the genes for all the blood groups We do not know on which pair any of them are but we do know that none of them are on the sex pair

It is unlikely that any of the genes for the other blood groups are located on the same chromosome pair as the Rh genes whichever it may be There is some fairly strong evidence from Copenhagen that one chromosome pair may carry the genes for two blood group systems Lutheran and Lewis

But wherever it is situated each blood group gene is strung amongst hundreds of other probably more important genes and by observing which characters are inherited with which blood group the position of more and more of these other genes will gradually be plotted—until we have chromosome maps for man such as we already have in detail for Drosophila and less completely for the mouse So much for blood groups as an instrument for the cartographical exploration of the germ plasm

Blood groups contribute to the science of genetics in another way There are probably but few steps between a blood group antigen and the gene that gives rise to it and in studying the nature of these antigens we are probably studying the nature of genes in the most direct way yet possible

I have taken a few minutes of your time to call to mind some of the wider aspects of blood groups because I feel we lose proportion if we think of them only in terms of transfusion and of hemolytic disease of the newborn Doubtless

Each Rh chromosome carries a gene for C or c for D or d, and for E or e It has been a matter of argument whether there are three separable genes separable but very close together or whether there is but one gene containing three inseparable sub genes These two possibilities are indicated diagrammatically of course in this chart There is some indirect evidence in favor of the three gene interpretation but the distinction between the two possibilities is not of any real practical importance

The chart shows the frequencies in England of the chromosome combinations and they are about the same in Argentina If say E is present it is much more likely that it is on a chromosome carrying a D than on a chromosome carrying a d so the presence of E can be used as an indicator of the presence of D Similarly C is much more likely on frequency grounds to be attached to a D than to a d so C like E serves as a fairly reliable indicator of the presence of D

Thus by using the four anti Rh sera now fairly widely available (that is anti D anti C anti c and anti E) we can make a fairly good guess at a genotype when there are no Rh negative children *to settle the question* (*chart 3*) Blood reacting ++++− could be one of two genotypes but since the frequency of one of them in the general population is 32 7% whilst the frequency of the other is only 2 2% the probability is that our sample is of the first genotype The error involved in this guess is 6% provided the sample of blood is from anyone who is not the father of children with hemolytic disease If as is very often the case the sample of blood is from the father of a child with hemolytic disease then a correction has to be made

The reason for the correction is this it is found empirically that such fathers are 1 75 times more often homozygous *DD* than is the Rh positive population as a whole The reason for this preponderance is clear two Rh positive fetuses

CHART 3 —*The Errors Involved when the Genotypes Are Guessed from the Reactions of anti C anti c anti D and anti E*

Reaction of blood with				First guess	Reasonably common alternatives	Approximate error	
Anti C	Anti c	Anti D	Anti E			(1) Unselected persons	(2) Fathers of children with hemolytic disease
						%	%
+	+	+	−	CDe/cde 32 7%	CDe/cDe 2 2%	6	21
+	−	+	−	CDe/CDe 17 7%	CDe/Cde 0 8%	4	1
−	+	+	+	cDE/cde 11 0%	cDE/cDE & cDE/cDe 2 7%	70	49
+	+	+	+	CDe/cDE 11 9%	CDe/cdE & cDE/Cde 1 3%	10	3

In the remaining 7 combinations the errors are all very small

It is still unknown why some women become immunized while others escape, nor is it yet known why a mother is less likely to make anti Rh if her husband's ABO group is incompatible with hers This protection is quite appreciable if an Rh negative woman marries an Rh positive man her risk of making anti Rh is reduced by a half if his ABO group is incompatible with hers

There is no new serologic method of distinguishing between heterozygous and homozygous Rh positive blood It is, as you know, the antigen called D or Rh_0 that is the cause of nearly all the trouble, and it is usually required to know whether the father of a child with hemolytic disease is homozygous DD or heterozygous Dd

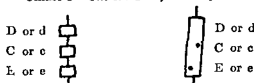
The antigen D behaves as a dominant mendelian character, and we cannot distinguish between DD and Dd by the use of anti D alone If anti-d were available (and unfortunately it is not), the problem would easily be solved anti d would agglutinate heterozygous Dd blood but not homozygous DD blood

Without the aid of anti d there are two ways of distinguishing between a heterozygote and a homozygote One way is certain the other enables us to make a good guess

Anyone who is Rh positive but who has an Rh negative dd parent or child must be heterozygous Dd for such a person must have received or have had handed on a d gene Thus by testing his parents or his previous children we can sometimes demonstrate with certainty that the father of a child with hemolytic disease is heterozygous Dd and this by the use of anti D anti Rh alone

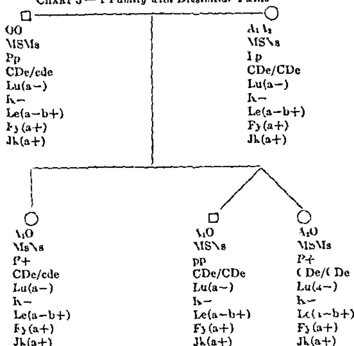
There is of course another way a way in which a very good guess can be made whether a person is heterozygous or homozygous for D for this we use the other recognizable antigens in the Rh complex (Chart 2) In England we call them C and c E and e, and the antisera which identify these antigens are now fairly widely available with the exception of anti e which is still possessed by only a few laboratories

CHART 2—*The Rh Groups in England*



8 chromosome combinations			36 genotypes the commonest being		
CDe	R_1	4205	CDe/cde	R_1r	32 68%
cde	r	3886	CDe/CDe	R_1R_1	17 76%
cDE	R_2	1411	cde/cde	rr	15 10%
cDe	R_0	0257	CDe/cDE	R_1R_2	11 86%
cdE	R	0119	cDE/cde	R_2r	10 94%
Cde	R	0098			
CDF	R	0024			
CdE	R_y	but a few found			

CHART 5 — A *Family with Dissimilar Twins*



In England the commonest combination of groups is O, MsNs P+ CDe/cde (R_1r) Lu(a−) kk Le(a− b+) Fy^a Fy^b and Jk(a+) But even this most common combination of groups occurs only once in 180 people

This high state of individuality impresses itself in another way In any one family it is rather unusual to find two children with exactly the same groups For example in the family shown in chart 5 the eldest child differs from the boy twin in three systems MNS P and Rh and from the girl twin in three systems ABO MNS and Rh The twins are of course dissimilar for they are of different sex but the blood groups show this also for the twins differ in their ABO MNS and P groups

Chart 6 shows a family in which two children have exactly the same group but this is because they are identical twins and since identical twins have identical genes the blood groups of such a pair must be the same

In this family the path of maternal and paternal genes to the children can be followed remarkably clearly For example the father has given

his A gene to both twins though he had an O gene to give
his Ms gene to both twins though he had an NS gene to give
his p gene to both twins though he had a P gene to give
his cde gene to both twins though he had a CDe gene to give
his K gene to both twins though he had a k gene to give
his Jk gene to both twins though he had a Jk gene to give

In the same way the mother has some alternative genes that she could have

are usually necessary for the stimulation of anti Rh The wife of a homozygous DD man is much more likely to have two Rh positive fetuses than is the wife of a heterozygous Dd man, half of whose children will, on the average be Rh negative dd

The necessary corrections have been applied in the last column

So you see most of the guesses are good ones and can be a real help in advising the parents in these families In the case of the reaction $-+++$, the guess cannot be made unless the sample is further tested with the anti e serum, which greatly reduces the error

The most recent blood group discoveries such as those of the Duffy and the Kidd systems have so far contributed more to genetics than to medicine though they are the occasional cause of acute clinical problems

Each new system increases in geometric proportion the number of blood group combinations known to be possible This is because the new divisions which it makes are independent of the divisions made by the other systems For example Duffy positive persons are as frequently Lutheran positive as are Duffy negative persons Kidd positive persons are as often Rh negative as are Kidd negative persons and so on and so on through all the permutations of the nine systems

Now I must apologize for I am off the track again and would like to spend the remaining minutes in speaking of the individuality of the blood

Chart 4 shows that with the antisera available to us we could distinguish more than 300 000 different kinds of blood and this is a very conservative estimate for I have not included some of the rarer allelomorphs such as A_3 M N D c and C Nor have I included another four systems the Levay the Gr the Miltenberger and the Jay I have left out these four systems for two reasons first far too few families have been tested to consider them on the same footing as the nine systems of the chart and secondly the frequencies of the two groups which each of the four systems defines are not such as to be of any practical use in genetics for almost no one has the first three antigens Levay Cr and Miltenberger while practically everyone has the fourth Jay

CHART 4—*The Blood Group Distinctions that Can Now Be Made in Some Laboratories*

Blood group system	Sera available	Number of recognizable phenotypes
A_1A_2BO	Anti A B A_1	6
MNSs	Anti M N S s	9
P	Anti P	2
Rh	Anti C c C D E e	26
Lutheran	Anti Lu	2
Kell	Anti K k	3
Lewis	Anti Le Le^b	3
Duffy	Anti Fy Fy^b	3
Kidd	Anti Jk	2

Phenotype combinations—303 264

of nine of their colleagues With the help of all the antisera discovered since that time our ability to distinguish between samples of blood has increased to such a degree that at the Lister Institute where I work just over 100 colleagues have been fully grouped and no two of them are exactly the same This means that if we were given an unlabeled sample of blood from anyone in the Institute we could identify the owner of the blood Indeed the day foreseen by Landsteiner when blood groups would be known to be as individual as finger prints has almost arrived

Grupos Sanguíneos y Genética

Hay ahora en el hombre nueve sistemas de grupos sanguíneos bien demostrados De estos se puede decir que únicamente los sistemas ABO y Rh son de primordial importancia en medicina clínica aunque de tanto en tanto los restantes siete sistemas pueden originar problemas agudos

Los nueve sistemas son de fundamental importancia en la genética humana En efecto ellos contribuyen con la mayor parte de lo poco que sabemos a propósito de la topografía de los genes en nuestros cromosomas Los genes de los grupos sanguíneos sirven como puntos fijos alrededor de los cuales se tiene la esperanza de ir haciendo el plano de las posiciones de más y más genes fisiologicos y patológicos

Los grupos sanguíneos son de importancia científica por otro motivo Ellos constituyen probablemente uno de los pocos pasos entre un antígeno de grupo sanguíneo y el gen que lo da origen Estudiando la naturaleza de ese antígeno se estudia la naturaleza de los genes de la manera más directa posible hasta ahora

El descubrimiento hecho por Levine y sus colegas de la causa primaria de la enfermedad hemolítica del recién nacido tuvo una consecuencia que no fué inmediatamente obvia el concepto de que cierta instrucción básica en genética fuera parte inevitable de una buena educacion médica

El diagnostico serológico de la enfermedad hemolítica ha progresado en los últimos cinco años más por causa del uso cada vez más extendido de las pruebas de la albúmina del suero antiglobulinico y de la tripsina que por el descubrimiento de alguna nueva técnica para la demostracion de anticuerpos Rh Nuestra capacidad para distinguir entre personas Rh positivas homocigoticas y heterocigoticas sigue en el mismo estado que en 1945 aunque la creciente disponibilidad de los anticuerpos necesarios ha hecho las pruebas de uso más general

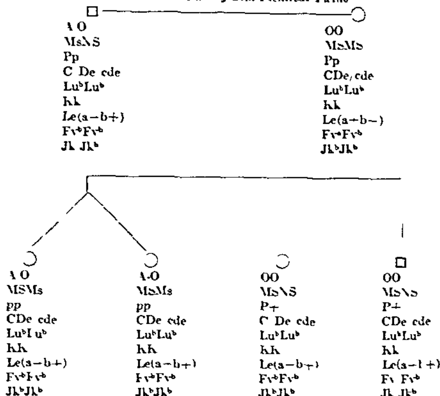
Parece ahora establecido que la enfermedad hemolítica puede ser causada por anti A y anti B más raramente por anti K anti k y anti s

Los grupos sanguíneos descubiertos más recientemente tales como los correspondientes a los sistemas Duffy y Kidd han contribuido hasta ahora más a la genética que a la medicina clínica

Cada sistema nuevo aumenta en proporcion geométrica el número de las posibles combinaciones grupales sanguíneas las cuales alcanzan ya a millones

En Inglaterra la combinacion más común se encuentra una vez cada 180 personas Justamente más de cien compañeros de tareas del Instituto Lister han sido agrupados completamente y no se ha encontrado dos de ellos que sean iguales Verdaderamente casi ha llegado el día previsto por Landsteiner en que los grupos sanguíneos llegarían a ser tan individuales como las impresiones digitales

CHART 6 — A *Family with Identical Twins*



given to the twins had they been dissimilar but yet again she always gave the same gene to both twins. She has given

her p gene to both twins though she had a P gene to give
her CDe gene to both twins though she had a cde gene to give
her K gene to both twins though she had a k gene to give
her Fy^b gene to both twins though she had an Fy^a gene to give

When all these chances are calculated we find that there is only 1 chance in 1000 that these twins so alike in sex and blood groups would be dissimilar.

I have shown you this chart in some detail not so much because of the twin diagnosis but to get you into the swing of how blood groups are inherited. It is really very simple. A parent has two genes for each group. These two genes may be the same or different. *One of the two is handed on to each child*, and which one of the two is handed on is determined purely by chance.

You will see that the groups are written in the order of their discoveries. In my Unit we keep strictly to this order because now that the list of any given individual's blood groups is so long we find *that a fixed order is essential* and the chronological seems the most reasonable.

In 1931 Landsteiner and Levine published a table showing that with the antisera then available they were able to distinguish between the blood of each

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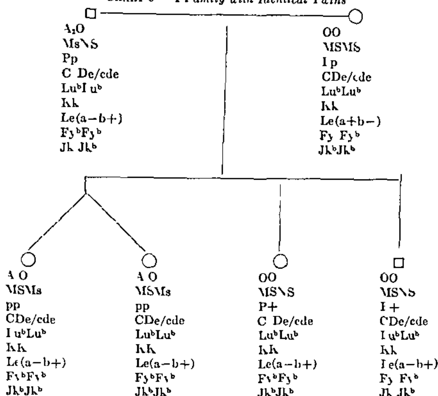


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You will see that the groups are written in the order of their discoveries In my Unit we keep strictly to this order because now that the list of any given individual s blood groups is so long we find that a fixed order is essential and the chronological seems the most reasonable

In 1931 Landsteiner and Levine published a table showing that with the antisera then available they were able to distinguish between the blood of each

immune globulins other than the true blockers. The characterization of anti-Rh immune globulins on the basis of their activities by various serological testing methods, their occurrence in different fractions of serum[12, 14] and their reaction to various physical and chemical reagents do not appear to us to allow for such a loose interpretation. From various publications[11, 16] as well as from the data to be presented here, it is evident that the agglutinin and the blocking agglutinoid are distinct entities. In addition, the agglutinoid (blocking antibody) is different both chemically and serologically from that group of immune globulins demonstrated with the albumin technic. These antibodies which neither block nor agglutinate constitute a third variety of antibodies as previously described[16, 18] and were designated cryptagglutinoids.

By using various antibody detection technics in the study of a number of sera containing anti-Rh antibodies, it was possible to demonstrate the presence of an Rh antibody that gave this third variety or cryptagglutinoid type of reactivity.[16, 18] Thus, the comparison of Rh antibody titers by such methods as saline agglutination, the original Wiener blocking test, the albumin test and the Coombs-Mourant and Race anti-human globulin test[17] revealed antibody patterns that were inconsistent with the concept that these reactions and titers were merely due to a difference in sensitivity of the technics employed.[11, 16, 17] It was evident that immune globulins were present in most human anti-Rh sera that differed from the previously described agglutinin and blocking antibody. These immune globulins, named the cryptagglutinoids, neither agglutinated in the presence of saline nor blocked the reaction between agglutinin and the Rh antigen. They did, however, specifically absorb on the red cell antigen and could be detected by the anti-human globulin test. In addition, most cryptagglutinoids can produce agglutination when the proper concentration of hydrophilic colloids are used instead of saline as the diluent. In figure 1 is seen the serological basis for our classification of the various anti-Rh immune globulins. It can be seen that a distinction can readily be made between the agglutinin and the agglutinoid on

ANTIBODY DESIGNATION	Tests characterizing heterogeneity of CDE cde antibodies					
	Agglutination in Saline	Blocking Test	Capillary Tube Agglutination	Agglutination in Bovine Albumin	Developing Test (Anti-Human Globulin)	Hemolysis
Classical Agglutinin	+	−	+	+	+	+
Agglutinoids (Blocking Antibodies)	−	+	−	+ or −	+	+?
Cryptagglutinoid	−	−	+	+	+	+
	−	−	−	+	+	+
	−	−	−	−	+	+

FIG. 1.—Characterization of CDE cde (Rh Hr) antibodies by differences in reactivity in various serologic tests

VI 4

Separation of Anti-Rh Agglutinins Agglutinoids and Cryptagglutinoids

SOL HABERMAN and JOSEPH M HILL*

DURING the early work on the detection of isoimmunization to the Rh antigens, the classic agglutination test utilizing saline suspended erythrocytes was employed[1,2] This approach to the demonstration of Rh antibodies failed to give results in many patients exhibiting clinical manifestations of isoimmunization[3-6] In spite of this inconsistency these reports were repeatedly confirmed In addition to such discrepancies many workers observed the lack of correlation between the titer of Rh agglutinins that were found and the severity of symptoms in the affected patients

However the demonstration of prozones in anti Rh sera began to shed new light on the isoimmunization problem Explanations for the zone phenomenon were offered in the separate reports of Wiener[7] Diamond and Abelson[8] and Race[9] of the blocking inhibitor or incomplete Rh antibody This antibody was characterized as capable of specific adsorption on and saturation of the Rh antigen without producing apparent agglutination From these findings Wiener devised the blocking test and labelled the antibody so detected as the blocking antibody This technic and the methods of employing bovine albumin[10] *human serum*[11] *or other hydrophilic colloids*[4] *as a diluent instead* of saline provided a means of detecting antibodies in general that were not demonstrable by the classical saline agglutination method

The description of such prozone producing antibodies is not entirely new As early as 1902 Eisenberg and Volk[1] using heated antisera demonstrated a zone reaction in an anti bacterial agglutinin These workers attributed this prozone to the presence of a heat modified agglutinin (named *agglutinoid*) that possessed the ability to adsorb on the antigen without producing clumping The role attributed to these agglutinoids is compatible with the characteristics of the blocking antibody although there is no direct evidence that the effect studied by Eisenberg and Volk is identical to the effect seen in antibodies of the Rh variety We have however adopted the older term agglutinoid and consider it synonymous with the term blocking antibody as originally used by Wiener But in view of the fact that the term blocking antibody has been very broadly interpreted by some workers as any antibody other than the agglutinin which produces clumping in the presence of adequate amounts of hydrophilic colloids we have felt that the original connotation of this term has been altered to cover

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particles, their solubility is largely dependent on the concentration and the nature of the ions of the solution. Thus they may be fractionated by adjustment of the electrolytic concentrations. All of the fractions isolated were related to the albumin, alpha, beta and gamma globulin components of human serum as depicted by electrophoretic patterns.[13]

The results of this fractionation on an Rh antibody containing sera showed that the gamma globulin contained the agglutinins. The agglutinoid ("blocking" antibody) was found mostly in the albumin and water-soluble globulin fraction. A small amount of specific blocking effect was present in the beta globulin with some traces in the alpha fraction. The beta globulin contained the remainder of the cryptagglutinoids that were not present in the albumin and water-soluble globulin fraction. Our findings and those of Witebsky et al. have been confirmed to an extent by the work of Cann, Brown, Gajdusek, Kirkwood and Sturgeon[4] using electrophoresis convection fractionation of anti-Rh sera. From this data it is possible that all of the serum globulins may contain anti-Rh antibodies.

The repeated physico-chemical separation of agglutinins, agglutinoids and cryptagglutinoids by three different groups of investigators is contrary to the viewpoint that the blockers (agglutinoids) and those immune globulins that produce clumping of erythrocytes in albumin suspension (cryptagglutinoid) are the same antibody. The apparent chemical as well as immunological independence of the types of Rh immune globulins led to the following additional physical chemical studies in order to further demonstrate the differences between the Rh immune globulins.

EXPERIMENTAL

Some Effects of Temperature. With the knowledge that heating to a critical temperature will destroy the Rh agglutinin,[25] experiments were designed to study the effects of heat on the three orders of reactivity of Rh immune globulins. Five cc. aliquots of anti-Rh sera of known immune globulin content (anti-C + D) were exposed to 30°C., 37°C., 56°C., 65°C. and 70°C. in controlled water baths for one hour each. After this treatment each specimen was tested for antibody activity through titrations by the following methods: agglutination in saline as the diluent, agglutination in 20 per cent bovine albumin, the blocking test, and the anti-human globulin test. The anti-C + D serum had a saline agglutinin for the C antigen only. The cryptagglutinoid and agglutinoid were anti-D. Consequently the test for the effects on the agglutinin were done with cells having the genotype Cde/cde and the observations on the agglutinoid were made with erythrocytes of the genotype cDE/cDE. In the saline agglutination test 0.85 per cent saline was used to prepare the 2 per cent erythrocyte suspensions and the titration dilutions of the sera. In the case of albumin titration, the 2 per cent erythrocyte antigens were suspended in 20 per cent bovine albumin (Armour) and all dilutions of serum were prepared in the same concentration of albumin. In the case of the blocking test, 0.85 per cent saline dilution of both erythrocyte and serum was used in the preparation of titrations. To one drop

the basis of the agglutination in saline and the Wiener blocking test The third group *is heterogeneous and contains at least* three types of serological activity however, they have been classified together as cryptagglutinoids until more can be learned of their immuno chemical nature

In a study of the antibody patterns found during immunization of Rh negative human volunteers as well as the natural immunization of Rh negative women by pregnancy it was found that the cryptagglutinoid was frequently the first antibody to appear in the sera[16] In some cases the saline agglutinin failed to attain more than minimal titers such as 1/8 or less, and in others the agglutinin appeared somewhat later in the immunization process but seldom attained the concentration found for the cryptagglutinoids The agglutinoid was consistently low rarely achieving a demonstrable blocking titer of 1/512 or higher The presence of the agglutinoid produced zones in the saline agglutinin titration Frequently these prezones also occurred in the albumin titration In some sera the blocking titer would exceed and completely mask the presence of the saline agglutinin thus giving the impression that agglutinins were not present in the serum however these could be demonstrated by fractionation of the serum From these observations it was felt that the classification of Rh antibodies on the basis of their appearance early or late in the immunization process was not justified

The fact that the Rh antibodies differed sufficiently in their serologic reactivity to allow for segregation into three classes suggested that there might be detectable chemical differences that could be exploited to further characterize and possibly separate these frequently found mixtures of immune globulins[18] The use of tannic acid as a selective serum protein precipitant[19] yielded results showing the serologic group of cryptagglutinoids were made up of at least two immune globulins one a water soluble protein and the other a salt soluble beta globulin In addition the agglutinoid was usually found in greatest concentration in the water soluble fractions

Although the first efforts to separate cryptagglutinoids from the other immune globulins by electrophoresis were inconclusive the data obtained showed anti body activity in the beta as well as the gamma globulin fractions of the serum[16] Prior to this publication Witebsky and Mohn[14, 0] reported the separation of a blocking antibody from saline agglutinins by a simple dialysis technic The blocking antibody was found mainly in the supernatant containing albumin and some globulin With further studies of this type Witebsky again demonstrated antibodies other than agglutinins and blockers[1] The use of the dialysis method in this laboratory yielded results that confirmed the findings described by Witebsky and Mohn[14, 20, 2]

Because of the uncertain control of saline concentration and pH in dialysis against distilled water a more precise method of serum fractionation was sought The Reid Jones[3] serum protein fractionation process employing ion exchange resins was found to be especially suitable for antibody separation In this method antibody containing sera were passed through a mixture of cation and anion exchange resins to remove the salts Since the antibodies are large colloidal

globulins In these studies the data obtained with 3 M urea gave rapid and clear results that were reversible and are herewith presented

Equal volumes of anti Rh sera and 10 M urea were mixed and allowed to stand at room temperature for one hour, and for 23 hours at 5°C Samples were withdrawn for antibody assay at 1 5, and 24 hours Each sample was tested by the saline agglutination test blocking test,' agglutination in 20 per cent bovine albumin and the anti human globulin test In this anti D serum there was a mixture of agglutinin, agglutinoid and cryptagglutinoids Consequently blood of the genotype cDe/cde was used as the antigen in the antibody assays

It was found that the presence of concentrated urea in the titrations caused hemolysis of the test erythrocytes in undiluted and 1/2 dilutions of the serum urea mixtures This effect disappeared in the 1/4 dilution This hemolytic effect was also found when the same concentration of urea was added to neutral AB serum and consequently it was interpreted to be due to the urea False agglutination and nonspecific interference with agglutination was not noted in the controls when the urea treated AB serum was tested against the erythrocyte antigen

At the end of 24 hours of exposure the serum urea mixtures were either dialysed against saline or treated with urease to remove the urea from the solution

In the case of dialysis the serum urea mixture was placed in a dialysis bag and immersed in a container holding one liter of saline The saline was changed frequently during dialysis The dialysis bag was attached to a motor driven cog wheel which turned at the rate of 30 rpm and thus kept the bag in continuous motion Under these conditions, the urea could be removed within 12 hours

In these experiments involving the removal of the urea by urease the following method was used To each 2 5 cc of serum urea mixture 1 cc of 10 per cent urease (Crystalline Jackbean origin) was added drop by drop The urease was thoroughly mixed with the serum as it was added As ammonia was released by the reaction the shift in pH of the serum mixture was readjusted to the original pH 7 5 with 0 1 N HCl When the urea was removed corrections were made for dilution by reagents The dilution usually established was 1/8

After removal of urea had been accomplished by dialysis or urease the resultant serum mixture was titrated by the saline agglutination method the blocking test agglutination in 20 per cent bovine albumin and the anti human globulin test

Results

The effects of temperature on the Rh immune globulins are presented in figure 2 It can be seen from the curves showing end points of titrations that the saline agglutinin shows marked denaturation beyond 56 C This effect is expected in view of the fact that gamma globulins are sensitive to such temperatures The agglutinoid however showed resistance to 65°C but beyond that temperature some denaturation occurred This denaturation and weakening of the blocking effect was not complete after 1 hour of treatment Even though the temperature was maintained for 2 hours at 65 C and coagulation of the serum occurred the

of each dilution of serum in a small tube was added 1 drop of a 2 per cent suspension of erythrocytes Rh negative cells in the presence of serum and Rh positive cells without antiserum were used as controls The mixtures were incubated at 37°C for 1 hour and observed for agglutination after centrifugation at 750-1000 rpm for 1 minute If prezones or weakness of reactivity was observed a drop of known saline agglutinin (anti D, titer 1/16) was added to each tube in the titration A second incubation for 1 hour at 37°C was then done, and the tubes again mildly centrifuged and observed for clumping If the anti D reagent failed to give strong clumping as shown by the controls the result was interpreted as a blocking effect The degree of interference was judged as follows 4+ blocking when no clumping was observed after the known saline agglutinin was added 3+ blocking when only small clumps of erythrocytes were observed, 2+ blocking when moderate sized clumps involving all of the test erythrocytes was present, 1+ blocking when large clumps but not complete agglutination (as compared with controls) was found

The saline agglutination and albumin titrations were performed in the usual manner using one drop of serum dilution to one drop of 2 per cent erythrocyte suspension After 1 hour of incubation at 37°C the tubes were centrifuged at 750-1000 rpm for 1 minute and observed for clumping The anti human globulin test titrations were performed as previously described [15, 17, 18]

The sera exposed to 65°C and 70°C showed considerable coagulation due to heat In these cases the coagulum was loosened and the fluid was expressed by high speed centrifugation (4000 rpm for 20 minutes) These fluids were tested for serologic activity in the above described manner

Some Effects of Alternation of pH The knowledge that the stability of globulins is influenced by the pH of its medium led to a series of experiments to determine the effects of alterations of pH on the three orders of Rh immune globulins

Five cc aliquots of the sera used in the above experiments were adjusted to pH 3 2 5 0 6 5 7 2 8 5 and 9 0 These were kept at 5 C for seven days At the end of this period the sera were adjusted to pH 7 2 using either 0 1 N HCl or 0 1 N NaOH and corrections were made to account for dilution These were then tested by saline agglutination the blocking test the reactivity in 20 per cent bovine albumin and by the anti human globulin test by the methods described above

Selective and Reversible Action of Urea on Antibodies The known action of urea on serum proteins led to the use of this reagent in these studies The work of Neurath and Saum [6] and that of Mirsky [7] has demonstrated that concentrated solutions of urea cause serum albumin to have an increased viscosity and a decreased diffusion constant This has been interpreted as indicative of a complete unfolding of the protein molecule Also removal of the urea by dialysis has indicated that the altered molecules refold in apparently a random manner with the albumin now present in a polydispersed aggregation [23] This action of urea on albumin led to the use of this reagent in these studies

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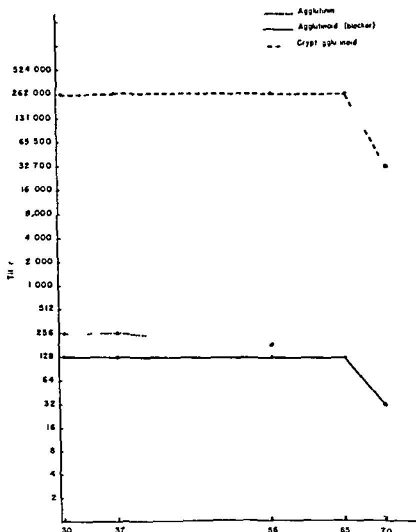


FIG 2A —Effect of heat (exposure time one hour)

This zone cannot be attributed directly to urea itself because the tubes showing this increased zone of inhibition gave agglutination when saline agglutinins anti c were added The test erythrocytes (cDe/cde) even in the presence of urea treated antibodies were clumped by the anti-c once the titration reached 1/4 In addition anti human globulin tests on these titrations in saline were positive to the same titer as the original saline agglutinin titer of 1/4 000 It was felt that these latter tests acted as additional controls on the experiments

When the urea was removed by dialysis or urease the denaturation of the immune globulins was largely reversed and the original titers of agglutinin agglutinoid and cryptagglutinoid was restored In the case of the agglutinin

Temperature	Test	Test cells
57° C	Saline agglutination	Cde/cde
	20% albumin	DE/cDE
	Blocking test	cDE/DE
56° C	Saline agglutination	Cde/cde
	20% albumin	cDE/cDE
	Blocking test	DE/cDE
65°	Saline agglutination	Cde/cde
	20% albumin	DE/cDE
	Blocking test	DE/cDE
70°	Saline agglutination	Cde/cde
	20% albumin	DE/cDE
	Blocking test	DE/cDE

Fig 2 —Effect of heat on Rh antibodies (titers after one hour heat treatment serum = anti C + D)

expressed fluids still retained the agglutinoid titer One hour at 70 C caused coagulation of the serum and produced a significant drop in agglutinoid titer to 1/32 The cryptagglutinoid gave a similar result when the results were graphed However, the loss of titer and strength of reaction of this immune globulin was significantly reduced

The data presented in figure 3 are representative of what is found when a potent anti CD serum is exposed to changes in pH for periods of time such as 7 days It can be seen that under alkaline conditions (pH 7 5, 8 5 and 9 0) no detectable change in titer or strength of reaction occurred On the other hand acid conditions were deleterious to both the agglutinin and cryptagglutinoid The agglutinoid in this case remained stable at a titer of 1/128 throughout the conditions of these studies The agglutinin was sensitive below pH 6 5 and was almost completely inactivated by pH 3 2 The cryptagglutinoid dropped in titer to 1/128 000 on exposure to the acid pH 3 2

The results of the experiments using 5 M urea on anti D sera of high titer were very significant (fig 4) Within one hour of the time the urea was added to the antiserum the ability of the saline agglutinin to produce clumping was almost lost the cryptagglutinoid lost titer and strength in the albumin titrations (1/64 000 to 1/32 000) but the agglutinoid increased in titer to 1 512 After 5 hours the agglutinin was inactivated and the cryptagglutinoid continued to lose both titer and strength of reaction the agglutinoid increased in blocking effect to a titer of 1/1000 At the end of 24 hours the cryptagglutinoid had been significantly weakened in both titer and reactivity and the agglutinin was still inactivated on the other hand the agglutinoid repeatedly showed a greater blocking titer rising from an original titer of 1/128 to 1/1 000 In addition the agglutinoid in the original serum showed a slight inhibition of clumping in the lower dilutions of the albumin titration (demonstrated up to 1/4) which was enhanced to produce a zone of inhibition that is observable in the 1/128 dilution

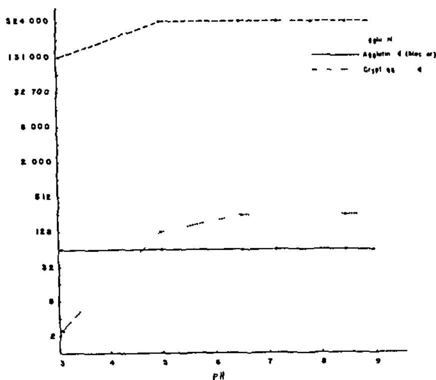


FIG. 3A—Effect of pH on Rh antibodies

of urea the activity of the agglutinoid increased Not only was this apparent by the increase in titer endpoints but the degree and strength of prezoning in the 20 per cent bovine albumin titrations was more pronounced In addition when the urea was removed the agglutinin and the cryptagglutinoid returned to approximately the original titer With this reactivation of antibody activity the competition with the agglutinoid was sufficient to depress the titer endpoint to its former level This demonstration of competitive effects of the various immune globulins is further evidence of their existence as entities Also this data is not compatible with the view that the agglutinoid (blocker) agglutinates the erythrocyte antigen in the presence of albumin It is difficult to accept the view that the differences observed in various serological tests on anti Rh sera are merely evidences of strength of titer or the physical features imposed by the methods *employed for their demonstration*

The possible mechanism of action of urea on the agglutinin and cryptagglutinoid may be found in the work of Mirsky Neurath and Saum Their studies indicate that urea acts to cause a progressive unfolding of the protein molecule The action is probably accomplished by the breaking of the hydrogen bonds of the helix of the *globulin molecules* The removal of urea allows the helix to reform From the studies presented in this paper the present authors feel that specificity and most of the reactivity of the immune globulins (agglutinins and

pH 3 2

CDe/cd
cDE/cDE
cDE/cDE

pH 5 0

Cd/cd
cDE/cDE
DE/DE

pH 6 5

Cde/cd
DE/DE
DE DE

pH 7 5

Cd/cd
DE DE
cDE/DE

pH 8 5

Cd/cd
cDE/cDE
cDE/cDE

pH 9 0

Cd/d
DE DE
DE/DE

Fig 3 —Effect of 7 day exposure to altered pH (anti C + D readjusted to pH 7 2 before titration)

some irreversible damage was evident because the clumping in saline was weaker and more easily dispersed as compared to the untreated serum controls The albumin titrations showed the same degree of clumping found in the untreated serum The specificity of the serum was unchanged

Discussion

When the results of these experiments are compared with each other there is evident an independence of reaction of the three orders of antibody reactivity In the case of the effects of temperature the agglutinin can be differentiated from the cryptagglutinoid and agglutinoid The studies of the effects of pH show the agglutinoid to have independent reactivity from that of the cryptagglutinoid The cryptagglutinoid showed only partial denaturation in acid This might be interpreted as indicative of its multiple nature that is the reaction in albumin menstruum may be due to more than one immune globulin of the cryptagglutinoid type

The results of the action of 5 M urea on anti Rh sera clearly demonstrate the competition between anti D agglutinins agglutinoids and cryptagglutinoid for the same antigen The graphic presentation of titers during urea treatment shows that as the saline agglutinin and cryptagglutinoid were depressed by the action

zación parcial en ácido Esto puede ser interpretado como signo de su naturaleza múltiple es decir la reaccion en medio albumino puede ser debida a algo más que a una globulina inmunizante del tipo criptaglutinoide

Los resultados de la accion de 5M urea sobre suero anti Rh demuestra claramente la competencia que existe para el mismo antigeno entre aglutininas aglutinoides y cripta glutinoides anti D La representacion gráfica de los títulos durante el tratamiento con urea muestran que al par que son deprimidas la aglutininas y criptaglutininas salinas por la accion de la urea la actividad del aglutinoide aumenta No solo este fenomeno resulta apreciable por el aumento en los títulos finales sino que tambien es más pronunciado el grado y la intensidad del fenomeno de prozona en las titulaciones en albumina bovina al 20% Además cuando la urea es eliminada la aglutinina y el criptaglutinoide vuelven aproximadamente al título original Con esta reactivación de la actividad del anticuerpo la competencia con el aglutinoide es suficiente para disminuir el título final a su primer nivel Esta demostracion de la competencia que existe entre la diversas globulinas inmunizantes es una nueva prueba de su existencia como entidades Tambien estos datos no son compatibles con el concepto de que el aglutinoide (bloqueador) aglutina el antígeno eritrocito en presencia de albumina Es difícil aceptar el concepto de que las diferencias observadas en diversas pruebas serologicas sobre suero anti Rh son simples signos del vigor del título o las características físicas impuestas por los métodos empleados para su demostracion

El posible mecanismo de la accion de la urea sobre la aglutinina y criptaglutinoide puede encontrarse en la obra de Mirsky Neurath y Saum Sus estudios indican que la urea causa un progresivo desdoblamiento de la molécula de proteina La accion se cumple probablemente por la rotura de las ligaduras del hidrogeno del helix de las moléculas de globulina La eliminacion de la urea permite reconstruirse al helix De los estudios presentados en dicho trabajo los autores de éste creen que la especificidad y gran parte de la reactividad de las globulinas inmunizantes (aglutininas y criptaglutinoides) se conservan pues la esencia de la molécula que lleva en sí la especificidad y reactividad del anticuerpo es poco afectada por la acción de la urea La urea no llega a atacar todas las globulinas inmunizantes En el aglutinoide se observa aumento de actividad debido posiblemente a la disminucion de la competencia de la aglutinina y criptaglutinoides

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BEFORE TREATMENT																			
SALINE AGGLUTINATION				[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			—	—	—	—	—	—	—
20% ALBUMIN	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]		—	—	—
BLOCKING TEST	[illegible]	[illegible]	[illegible]	[illegible]			—	—	—	—	—	—	—	—	—	—	—	—	—
UREA HR																			
SALINE AGGLUTINATION	hem.	—	—	—	—	±	[illegible]		—	—	—	—	—	—	—	—	—	—	—
20% ALBUMIN	hem.	—	—	—			[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			—	—	—	—	—
BLOCKING TEST	hem.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			—	—	—	—	—	—	—	—	—	—
UREA 3 S																			
SALINE AGGLUTINATION	hem.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
20% ALBUMIN	hem.	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]				—	—	—	—	—
BLOCKING TEST	hem.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			—	—	—	—	—	—	—	—	—
5 UREA 24 HRS																			
SALINE AGGLUTINATION	hem.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
20% ALBUMIN	hem.	—	—	—	—	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			—	—	—	—	—	—	—
BLOCKING TEST	hem.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		—	—	—	—	—	—	—	—	—
AFTER DIALYSIS																			
SALINE AGGLUTINATION	—	—				[illegible]	[illegible]		[illegible]				—	—	—	—	—	—	—
20% ALBUMIN	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		—	—	—
BLOCKING TEST	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			—	—	—	—	—	—	—	—	—	—	—	—

FIG 4 —Reversible effects of 5 molar urea on anti Rh serum (serum = anti D antigen = cDe/cde)

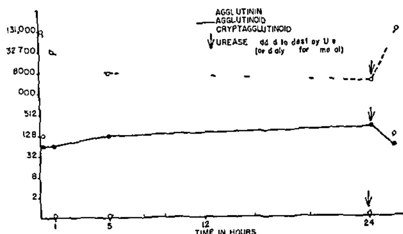


FIG 4A —Action of urea on Rh antibodies (concentration of urea = 5M)

cryptagglutinoids) are retained because the end plates of the molecule which carry the specificity and reactivity of the antibody are little affected by the action of urea Urea failed to attack all of the immune globulins In the agglutinoid there is increased activity due possibly to the depression of competition from the agglutinin and cryptagglutinoids

SEPARACION DE AGLUTININA AGLUTINOIDES Y CRIPTAGLUTINOIDES ANTI RH

Cuando se comparan entre sí los resultados que el autor refiere se comprueba una independencia de la reacción de los tres tipos de reactividad de anticuerpos En el caso de los efectos de la temperatura la aglutinina puede ser diferenciada del criptaglutinoide y el aglutinoide Los estudios de los efectos del pH muestran que el aglutinoide tiene una reactividad independiente de la del criptaglutinoide El criptaglutinoide muestra solo desnaturali

zación parcial en ácido. Esto puede ser interpretado como signo de su naturaleza múltiple, es decir, la reacción en medio albuminoso puede ser debida a algo más que a una globulina inmunizante del tipo criptaglutinoide.

Los resultados de la acción de 6M urea sobre suero anti-Rh demuestran claramente la competencia que existe para el mismo antígeno entre aglutininas, aglutinoides y criptaglutinoides anti-D. La representación gráfica de los títulos durante el tratamiento con urea muestran que al par que son deprimidas la aglutininas y criptaglutininas salinas por la acción de la urea, la actividad del aglutinoide aumenta. No solo este fenómeno resulta apreciable por el aumento en los títulos finales, sino que también es más pronunciado el grado y la intensidad del fenómeno de prozona en las titulaciones en albúmina bovina al 20%. Además, cuando la urea es eliminada, la aglutinina y el criptaglutinoide vuelven aproximadamente al título original. Con esta reactivación de la actividad del anticuerpo la competencia con el aglutinoide es suficiente para disminuir el título final a su primer nivel. Esta demostración de la competencia que existe entre las diversas globulinas inmunizantes es una nueva prueba de su existencia como entidades. También estos datos no son compatibles con el concepto de que el aglutinoide (bloqueador) aglutina el antígeno eritrocito en presencia de albúmina. Es difícil aceptar el concepto de que las diferencias observadas en diversas pruebas serológicas sobre suero anti-Rh son simples signos del vigor del título o las características físicas impuestas por los métodos empleados para su demostración.

El posible mecanismo de la acción de la urea sobre la aglutinina y criptaglutinoide puede encontrarse en la obra de Mirsky, Neurath y Saum. Sus estudios indican que la urea causa un progresivo desdoblamiento de la molécula de proteína. La acción se cumple probablemente por la rotura de las ligaduras del hidrógeno del helix de las moléculas de globulina. La eliminación de la urea permite reconstruirse al helix. De los estudios presentados en dicho trabajo, los autores de éste creen que la especificidad y gran parte de la reactividad de las globulinas inmunizantes (aglutininas y criptaglutinoides) se conservan, pues la esencia de la molécula que lleva en sí la especificidad y reactividad del anticuerpo es poco afectada por la acción de la urea. La urea no llega a atacar todas las globulinas inmunizantes. En el aglutinoide se observa aumento de actividad debido posiblemente a la disminución de la competencia de la aglutinina y criptaglutinoides.

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VI communication 1

Treatment of Hemolytic Disease of the Newborn

HORS BRESMEZ*

1 *Treatment of the Child*

We have employed a procedure that has given us excellent results in the treatment of hemolytic disease of the newborn when a live fetus is obtained and when the case is not very severe (when the case is severe, of course the treatment of choice is exchange transfusion). Our treatment consists of obtaining 10 cc. of blood from the father and injecting it subcutaneously to the baby in 10 different places, causing in this way a series of hematomas in which there is a concentration of paternal erythrocytes which will absorb a large amount of agglutinins from the child's circulation.

2 *Treatment of the Mother*

Antigen competition. Various cases have been reported in the literature as the one of Malaguzzi Valeri, in which anti typhoid vaccination was used for an antigenic competition in Rh negative pregnant women. *There is no doubt that the most effective antigenic competition* is that caused by saliva of opposite group (ABO system) secretor individuals. By this technique the production of anti Rh antibodies is considerably diminished and sometimes inhibited. The technique is very simple and not at all bothersome for the mother if we consider the splendid results obtained, above all in women married to individuals of their own group, because those of opposite group may already have a certain antigenic competition.

Technique. The pregnant Rh negative mother is injected subcutaneously with half a cc of sterile centrifuged saliva of a secretor individual of opposite group. This can also be done by injecting intradermally 0.1 cc. of the same substance, the injection being repeated in one week increasing the dose to 0.2 cc. if using the subcutaneous method. If the first dose gives a marked reaction, one must continue with the same dose, and if the reaction has been insufficient, the dose is repeated twice weekly during the rest of pregnancy. With this technique we have obtained live babies in women Rh negative who have previously had repeated abortions.

TRATAMIENTO DE LA ENFERMEDAD HEMOLÍTICA DEL RECIÉN NACIDO

1 *Tratamiento en el niño*

Hemos empleado un procedimiento que nos ha dado excelentes resultados en el tratamiento de la E. H. cuando se obtiene un feto vivo y no se trata de casos graves, en que como es sabido el tratamiento de elección es la exsanguíneo transfusión.

Consiste dicho tratamiento en la extracción de 10 c.c. de sangre del padre e inyectarla rápidamente en diez inyecciones subcutáneas al niño, provocándole una serie de hematomas en los que hay un acúmulo de hematíes paternos y que absorberán una gran cantidad de aglutininas que restarán de la circulación general.

2 *Tratamiento en la madre*

Competencias de antígenos. Aunque ya se han citado casos como los de Malaguzzi Valeri en los que se ha empleado vacuna antitífica como competencia antigénica en el organismo de mujeres Rh negativas creando anticuerpos inofensivos para el feto, no cabe duda que la competencia antigénica más eficaz es la provocada por saliva de grupo contrario con lo que la producción de anticuerpos anti Rh está disminuída y hasta suprimida.

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upon the curve of the maternal antibodies is discussed. All the babies from these mothers developed benign forms of hemolytic disease.

Considering the notable effects obtained with cortisone in the treatment of acquired hemolytic disease, it is indicated for both treatment and prevention of hemolytic disease of the newborn. The different hypotheses to explain the mechanism by which cortisone acts are discussed.

The results obtained by the author suggests the use of this method in a greater number of cases so as to arrive at definite conclusions.

VI communication 3

Treatment of Hemolytic Disease of the Newborn by Exsanguino Transfusion

G. BOMCHIL and M. MARCULIS*

44 cases of hemolytic disease of the newborn through sensitization by anti D and 1 case of anti L were treated. *The exsanguino transfusion was only carried out within thirty hours of birth in cases with manifest symptoms or with high agglutinin titer (1/16).* The high incidence of sensitization through transfusions (33%) and of previous fetal mortality (35%) explains the mortality rate of 27% of this series, which coincides with the *mortality rate observed by other authors in analogous circumstances. We also coincide* in the excellent results obtained in the very serious cases. There was no operative mortality. All surviving children were cured without nervous sequelae. 4 very serious cases born by cesarean section were cured. 2 cases died because of ruptured spleen or liver from obstetrical causes. *In 3 cases great meteorism appeared post operatively*; 2 were cured, ascites was found in the third. The best results were obtained in babies treated at birth owing to prenatal diagnosis (83% success). Save in four cases, the umbilical cord was used, with the open system and without heparinizing the baby.

The treatment of some of the mothers with haptens, antihistaminics and vaccines failed, as also the treatment of some of the babies with ACTH, and in 41 cases treated with transfusions 10% developed nervous sequelae.

Exsanguino transfusion is the treatment of choice in nearly all cases of opportunely diagnosed hemolytic disease of the newborn.

El Tratamiento de la Enfermedad Hemolítica Neonatal por la Exsanguíneotransfusión

Se trataron 44 casos de E. H. N. por sensibilización anti D y 1 por anti L. Solamente se practicó la exsanguíneotransfusión antes de las 30 horas en casos con síntomas manifiestos o bien con título aglutinante alto (1/16). La alta incidencia de sensibilización por transfusión (33%) y de mortalidad fetal previa (35% de los casos) explica la mortalidad de 27% de esta serie, coincidiendo con la observada en circunstancias análogas por otros autores. También coincidimos en los excelentes resultados observados en casos gravísimos. No hubo mortalidad operatoria. Todos los sobrevivientes curaron sin secuelas nerviosas hasta ahora. 4 casos gravísimos extraídos por cesárea curaron. 2 casos murieron por ruptura de bazo o hígado de causa obstétrica. En 3 casos apareció gran meteorismo postoperatorio, curando 2, encontrándose ascitis en el otro. Los mejores resultados se obtuvieron en casos

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La técnica es muy sencilla y poco molesta para la madre si se tienen en cuenta los magníficos resultados obtenidos sobre todo en mujeres casadas con individuos de su mismo grupo puesto que las de grupo contrario llevan ya en sí o pueden llevar una cierta competencia antigénica

Técnica Con saliva estéril y centrifugada de secretor de grupo contrario se inyecta a la madre gestante Rh negativa medio centímetro cúbico intramuscularmente o bien una décima intradermicamente repitiendo la inyeccion a la semana aumentando la dosis a 1 cc en el primer caso y a 2 décimas en el segundo Caso de habérsele producido una gran reaccion con la dosis anterior se debe insistir en la misma dosis y si la reaccion ha sido insuficiente repetir la dosis dos veces en semana durante todo el embarazo

Con esta tecnica hemos conseguido un feto vivo en mujeres Rh negativas con abortos habituales

VI communication 2

La Cortisona en el Tratamiento y Profilaxis de la Enfermedad Hemolitica del Recien Nacido

H LINARES GARZON*

Se presenta una serie de 11 niños con enfermedad hemolítica del recién nacido tratados con Cortisona en dosis de 25 mgs repetida de 3 a 7 veces con intervalos de 8 y 12 hs y transfusiones de globulos rojos sedimentados Fallecio un niño (18 18%) Los 10 restantes curaron evolucionando en forma extraordinariamente benigna a pesar de la gravedad inicial y de la isoinmunizacion materna

En una segunda serie se presentan 7 niños cuyas madres fueron tratadas con pequenas dosis de Cortone durante distintos periodos antes de finalizar el embarazo Fallecio un niño por una causa intercurrente (inyeccion subcutánea de 80 cc de suero glucosado al 50%) y otro a causa de la enfermedad hemolítica Los 5 restantes desarrollaron formas extraordinariamente benignas

En 5 de las madres tratadas se comprobó un marcado descenso del título de anticuerpos anti Rh atribuible a la accion de la Cortisona En una madre el título no vario y en otra se comprobó un aumento lo que justificaría la necesidad de utilizar dosis mayores 200 o mas mgs diarios cuando las dosis pequeñas no demuestran efectos favorables

Se discute la forma de acción de la Cortisona y se manifiesta que es necesaria una mayor experiencia para sentar normas definidas sobre dosificación de la droga

La mortalidad sobre los 17 casos (excluído 1 que fallecio por intercurrencia) fué de 2 niños (11 76%) y no hubo ningun caso con secuelas de ictericia nuclear

CORTISONE IN THE TREATMENT AND PROPHYLAXIS OF HEMOLYTIC DISEASE OF THE NEWBORN

A series of newborn babies with hemolytic disease due to maternal Rh isoimmunization is presented they were treated with transfusions of sedimented red cells and cortisone with excellent results All the babies developed benign forms of the disease which were cured without sequelae

A series of cases is presented of Rh negative sensitized mothers who were submitted to treatment with cortisone during the last three months of pregnancy The effect of cortisone

Instituto de Maternidad de la Facultad de Medicina de Cordoba e Instituto Nacional de Puericultura con la colaboración del Instituto Municipal de Hemoterapia de la ciudad de Córdoba Argentina

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* Policlínico Ramos Mejía y Argerich, Buenos Aires, Argentina

tratados al nacer merced al diagnóstico *prenatal* (83% de exitos) Salvo en 4 casos se uso la via umbilical con sistema abierto sin heparinizar al niño

Fracaso el tratamiento de algunas *madres con antihistamínicos* haptenes y vacunas y de algunos niños con ACTH y se registro 10% de secuelas nerviosas en 41 casos tratados por *transfusiones*

La exsanguíneotransfusion es el tratamiento de eleccion en casi todos los casos de E H N diagnosticados oportunamente

VI communication 4

On the Calculation of Race Mixture

F OTTENSOOSER*

When two populations (1 and 2) are mixed and the frequencies of *a gene in the three* populations are known ($p_1 > p_m > p_2$) the mixture degree (x) is very simply found by comparing the difference between the two parental populations with the difference between one of them and the mixed one in symbols

$$x = (p_m - p_2)/(p_1 - p_2)$$

will be the percentage of population 1 in the mixture Theoretically any gene of the three series should yield the same result However this did not happen in applications of the formula to Brazilian and other populations since 1944 While the mixture degrees for instance obtained from the genes A and O agreed satisfactorily the *value derived from* gene B did not even with gene frequencies adjusted (Boyd 1949) by the formulae of Bernstein (1930) or Stevens (1938)

The principal source of error is another one The mixture degree is likely to be wrong if the difference between the two parental populations is very small As shown by elementary statistics chance variations decrease as values decrease but to a lesser extent thus the chance variation of a small difference in gene frequency will be relatively greater than that of a great difference presented by another gene in the same series

In observations especially on whites Negroes and mulattoes of Sao Paulo the mixture degrees derived from enormous ABO series but with small differences in gene frequency did not fit whereas the values obtained from the genes R^0 and R^1 of small series but with strong differences fitted rather well

In several practical cases studied three series each of 100 to 700 individuals failed to give reliable values for the mixture degree unless the difference in gene frequency between the two parental populations exceeded 15 per cent

Sobre el Cálculo de Mezcla de Razas

Cuando dos poblaciones (1) y (2) se mezclan y la frecuencia de un gen en las tres poblaciones es conocida ($p_1 > p_m > p_2$) el grado de mezcla (x) es fácilmente encontrado comparando la diferencia entre las dos poblaciones paternas con la diferencia entre una de ellas y la mezclada en símbolo

$$x = (p_m - p_2)/(p_1 - p_2)$$

será el porcentage de la población 1 en la mezcla Teoricamente cualquier gen de las tres series debe dar el mismo resultado Sin embargo esto no aconteció al aplicar la fórmula

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en poblaciones brasileras y otras desde 1934. En cuanto que los grados de mezcla obtenidos por ejemplo de los genes A y O concordaron satisfactoriamente, el valor derivado del gen B no concordó mismo con frecuencias génicas ajustadas (B y d 1919) por las fórmulas de Bernstein (1930) o Stevens (1938).

La principal fuente de error es otra. El grado de mezcla puede fácilmente ser errado si la diferencia entre las dos poblaciones parientes es muy pequeña. Como lo muestra la estadística elemental, con valores decrecientes las variaciones casuales disminuyen también pero mucho menos, así la variación casual de una pequeña diferencia de la frecuencia de un gen será relativamente mayor que aquella de una gran diferencia presentada por otro gen en las mismas series.

En observaciones especialmente en blancos, negros y mulatos de Sao Paulo los grados de mezcla derivados de grandes series ABO, pero con pequeñas diferencias de frecuencias de genes, no concordó en cuanto que valores obtenidos de los genes R^o y R^1 de pequeñas series pero con fuertes diferencias concordaron bastante bien.

En varios casos prácticos estudiados las tres series en la una de 100-200 individuos más o menos, no dieron valores útiles para el grado de mezcla, al menos que la diferencia en la frecuencia génica entre las dos poblaciones parientes excediera el 10%.

VI communication 5

Comparacion de los Iso anticuerpos en el Suero, Calostro y Plasma del Cordon

F. OTTENSOOSER, Y. ERSIAN, R. PASCUALIN y R. FARIA*

Teniendo presente que el título de anticuerpos es más débil en el calostro que en el suero de la madre, mientras que ocurre lo contrario con las aglutininas anti A y anti B, el autor ha estudiado el paso de anticuerpos Rh, A y B en el calostro de dos mujeres O sensibilizadas a Rh_o con hijos de grupo O y en tres mujeres Rh positivas con potentes aglutininas anti A o anti B e hijos que sufrían enfermedad hemolítica.

El autor deja sin respuesta la cuestión de si el calostro con alto valor anti A o anti B puede contribuir a la enfermedad hemolítica, si bien existen algunas pruebas de que la vía intestinal debe funcionar para los anticuerpos maternos tanto en la especie humana como en varios animales.

En una mujer del grupo O sensibilizada por Rh (D) con un hijo eritroblastósico del grupo O fueron titulados el suero y el calostro en solución salina y albuminosa con células O Rh positivas, A Rh negativas y B Rh negativas. En otra mujer también del grupo O y sensibilizada por Rh (D) cuando tuvo hijo Rh negativo del grupo O fueron comparados del mismo modo calostro y plasma del cordón. El calostro del segundo día después del parto contenía menos anti Rh (D) pero más anti A y anti B que el suero de la madre o el plasma del cordón. En los siguientes días el valor en el calostro bajó, bajando más rápidamente el título en la solución albuminosa que en la salina.

En tres mujeres sensibilizadas por A o B persistieron valores muy altos anti A y anti B (16 000–20 000) en su leche una y dos semanas después del parto. En 1 de los tres casos la elevación se refería particularmente al título salino, varios meses después los títulos se invirtieron siendo mayores en albúmina que en salina. Los tres recién nacidos eran ictéricos y gravemente enfermos probablemente como resultado de la incompatibilidad A B. No se sabe si un valor extremadamente alto de valores anti A o anti B del calostro puede causar mal en los primeros días del nacimiento.

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COMPARISON OF ISOANTIBODIES IN SERUM COLOSTRUM AND CORD PLASMA

In a woman of group O sensitized by Rh_0 (D) with an erythroblastotic baby of group O serum and colostrum were titrated in saline and albumin with O Rh positive A rh negative and B rh negative cells In another woman also group O and sensitized by Rh_0 (D) after delivery of an rh negative child of group O colostrum and cord plasma were compared in the same way Colostrum of the second day after delivery contained less anti Rh_0 (D) but more anti A and anti B than the mother s serum or the cord plasma During the next few days the values of the colostrum dropped the albumin titer decreasing more quickly than the saline titer

In three women sensitized by A or B very high anti A or anti B values (15 000–20 000) persisted in their milk one to two weeks after delivery In two of the three cases the saline titer was the higher but after several months the order reversed the albumin titer be coming the higher The three newborns were icteric and severely ill probably as a re ult of the A B incompatibility It is still an open question whether or not extremely high anti A and anti B values of colostrum do harm during the first few days after delivery

VI communication 6

Los Grupos Sanguineos en la Poblacion de Bahia

MENANDRO NOVAIS*

Se presentan los resultados de la investigacion de los grupos ABO y Rh en blancos mestizos y negros de la poblacion de Salvador Bahia reclutados entre los dadores del Servicio de Transfusión de Sangre (S T S) y enfermos atendidos por este Centro de Hemo terapia en el curso de cinco anos Se hace un análisis estadísti o de la evolucion demo gráfica de la ciudad demostrando que el material estudiado corresponde numéricamente a los contingentes raciales que lo integran La frecuencia de los genes demuestra que el gen p disminuye progresivamente partiendo de los blancos mestizos y negros mientras que los genes q y r aumentan en la misma proporcion y en el mismo sentido La frecuencia del factor Rh disminuye en menor escala partiendo de los blancos (13 69%) mestizos (13 23%) y negros (11 78%) debiéndose hacer notar que nuestros índices de Rh en los negros son relativamente altos en comparación con los obtenidos por otros investigadores lo que indica la extension del mestizaje existente Se calcula el grado de mezcla racial admitiéndose la presencia de 62% de contingente negro en nuestros mestizos examinados Se presenta finalmente un cuadro que contiene el cálculo de los diferentes índices sero logicos a los cuales no se atribuye valor científico modernamente

BLOOD GROUPS IN THE POPULATION OF BAHIA

The results of the investigation of blood groups (ABO and Rh systems) on white half breed and Negro individuals of the population of Salvador (Bahia) in Brazil are presented in this work

A statistical analysis of the demographic evolution of the population of this city is made showing that the studied material corresponds numerically to the different racial groups pre ent The study of gene frequency demonstrates that the gene p diminishes progressively from white to half breed to Negro while the genes q and r increase in the same proportion and order The frequence of the Rh factor diminishes in a minor scale in the following order whites 13 69% half breed 13 23% and Negroes 11 78% Our

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Rh percentages in Negroes are relatively high in comparison with those obtained by other investigators which shows the extension of the present half breeding. The degree of racial mixture is calculated on the basis of a 62% Negro incidence in our examined half breeds. Finally a graph is presented containing the calculation of the different serological indices to which no scientific value is attributed at present.

VI communication 7

El Problema de la Nomenclatura del Factor Rh

C. MUÑOZ BARATTA*

Desde el descubrimiento del factor Rh por Landsteiner y Wiener y del Hr por Levine se han publicado numerosos trabajos sobre este tema para explicar una serie de fenómenos atribuídos a dichos factores.

Se han originado entusiastas discusiones acerca de las nomenclaturas propuestas por Wiener y por Fisher porque ambos pretenden la primacía de la suya.

Wiener reclama la prioridad y prueba en reciente publicación la conveniencia de usar los términos Rh Hr porque en su opinión son más enfáticos y fáciles de entender.

Fisher sostiene que las letras DCE/dce son mucho más simples y ha logrado la recomendación del Congreso Internacional de Hematología que se reunió en Buffalo en 1948.

Estudiando ambas nomenclaturas hemos concluído que le asiste a Wiener la prioridad por ser el descubridor del factor Rh y porque con este nombre es conocido en todos los medios científicos, además porque con esa sinonimia la explicación es más simple y comprensible.

Si se consideran simplemente los nombres de Rh rh' y rh'' se encontrará que es más fácil llamarlos D C E respectivamente como sostiene Fisher, pero si incluímos el factor Hr solo se establecería la diferencia viéndolos escritos o indicando después de cada letra si es mayúscula o minúscula, sobre todo tratándose de los genotipos, cuya lectura origina un verdadero enredo. Este método aparentemente más sencillo causa grandes confusiones entre los médicos no especializados que encuentran sumamente complicado el estudio del factor Rh, pues deben además tener en mente que D corresponde al Rh, C al rh' y E al rh'', etc.

Ahora bien, volviendo a la nomenclatura de Wiener observamos que las combinaciones del Rh_0, rh', rh'' forman nuevas entidades que él denomina Rh', Rh'', Rhy y Rhz, hecho que indudablemente trae cierta dificultad al lector poco experimentado y lo que se persigue en la ciencia es encontrar las cosas fáciles y al alcance de todo médico cualquiera sea su especialidad. Esta razón nos ha determinado a introducir una ligera modificación en la nomenclatura de Wiener a fin de hacerla más accesible a cualquier principiante.

Todo esto se puede simplificar con una sencilla fórmula resultante de la observación de que los factores Rh y Hr tienen como común denominador los sufijos (o) (') ('') que sólo pueden estar presentes en uno de ellos, faltando en el otro y vice versa, de suerte que cuando estén en el Rh no los portará el Hr y al revés, quiere decir que si representamos el Rh por la letra R y el Hr por la H seguidos por los sufijos (o) (') ('') el problema queda simplificado.

En esta forma se respeta el nombre del Rh que reclama Wiener con tanta justicia.

Puede abreviarse aun más la lectura mencionando tan solo al factor R con su sufijo y tratándose de los genotipos bastará con indicar si son homo o heterozigotas y su ubicación correspondiente.

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The Problem of Rh Nomenclature

The author prefers the Rh nomenclature of Wiener rather than the system adopted by Fisher To avoid complications in its reading he suggests a simple formula based on the observation that the factors Rh and Hr have as a common denominator the symbols (o) ( ) ( ) which can only be present in one of them and lacking in the other and vice versa If we represent the Rh by the letter R and the Hr by the letter H followed by the symbols (o) ( ) ( ) the problem would be simplified

VI communication 8

Tecnica Rápida de Titulación de Anticuerpos Rh Incompletos en Placa (Slide Test)

NESTOR F M PAGNIEZ*

La mayor responsabilidad de los anticuerpos incompletos en los procesos de vulnerabilidad clínica hace que sea especialmente importante el control periodico cuantitativo tanto para apreciar la evolucion como el pronóstico en los problemas de isohemosensibilizacion

Numerosas son las técnicas propuestas tales como las citadas por Bessis De Gowin Mourant Van Loghem Etcheverry Elliot etc siendo las dificultades mas comunes observadas en la práctica diaria las que a continuacion enumeramos

1 *Mediciones con cuenta gotas* Dejan mucho que desear en cuanto a la exactitud con el inconveniente que agregan la multiplicacion del error proporcional a la dilución lograda

2 *Mediciones con pipetas graduadas precisas cambiando cada pipeta en cada dilucion* Supone un gasto elevado de material máxime cuando sean numerosas las determinaciones a realizar

3 *Factor tiempo* Incubaciones de 30 a 60 y a 37 C seguidas de centrifugaciones en serie para las lecturas

Con la finalidad de facilitar este tipo de investigacion proponemos el empleo de titulaciones por dilucion directa del suero en examen en placa con control rápido por la prueba de Diamond Abelson

La prueba de Diamond Abelson siguiendo a Elliot puede denominarse como fundamental Ejecutada en condiciones correctas se nos presento siempre como notablemente simple rápida y economica mostrando una sensibilidad igual y en oportunidades superior a la de las pruebas en tubo

Empleamos una micro pipeta especial que permite la correcta medicion y diluciones de cantidades de 0 05 ml de suero y directamente en la placa o en los tubos donde se efectúe el examen

A continuacion se detalla la técnica rápida de titulacion de anticuerpos incompletos propuesta

Las diluciones geométricas del suero en examen se realizan en suero humano AB 4 partes mas albumina bovina Armour o Proviet una parte Se incluye un control del suero AB y de la sangre test

Previo calentamiento de la placa en el viewing box (40 a 50C) se procede al agregado directo de la sangre total test completando e la lectura hacia los 2

Se exponen asimismo las causas que pueden dificultar los resultados correctos

Laboratorio de Serología del Instituto Nacional de Hemoterapia Buenos Aires Argentina

Resumen lo

Presentamos una técnica rápida de titulación de anticuerpos incompletos accesible por su simplicidad y por el escaso requerimiento de material a los más modestos laboratorios

El empleo de una micro pipeta diluidora posibilita la correcta medición y dilución de cantidades de 0 05 ml de suero y directamente en la placa o en los tubos donde se efectúe el exámen

Exigencias de tiempo menos de 10

Se logra la adaptación cuantitativa de la técnica de Diamond Abelson con las ventajas de su rapidez y economía así como de su sensibilidad en oportunidades mayor que la de las pruebas en tubos

A RAPID SLIDE TECHNIQUE FOR TITRATION OF INCOMPLETE RH ANTIBODIES

We present a rapid technique for titration of incomplete antibodies accessible because of its simplicity and the small amounts of material required for the most modest laboratories The use of a diluting micro pipette allows the correct measurement and dilution of quantities of 0 05 ml of serum directly in the slide or in the tubes where the test is performed The time requirement less than 10 minutes

This is a quantitative adaptation of the Diamond Abelson technique with the added advantages of rapidity and economy Its sensitivity is sometimes more marked than in the commonly used tube tests

VI communication 9

Estudio Electroforetico y Polarografico de la Sangre de los Niños Afectos de Enfermedad Hemolitica

MANUEL HERRERO*

Se hace un estudio electroforético y polarográfico de 25 niños afectos de eritroblastosis fetal (E F) y de sus progenitores De este estudio se deduce

1 Hay un estrecho parecido entre el espectro electroforético de las proteínas del padre y del hijo

2 Polarográficamente se comprueba también un comportamiento parecido de las proteínas del padre y del hijo ante los agentes desnaturalizantes (pepsina y urea) como así mismo una cierta labilidad proteica de ambos con relación a la madre

3 De aquí se conjetura si esta labilidad proteica no se limita solamente a las proteínas séricas sino también a la globina eritrocítica De esta manera podrían explicarse todos aquellos casos que pudieron ser una E F no lo son y de aquellos otros que siéndola no lo debieron ser con arreglo a la teoría etiopatogénica general

ELECTROPHORETIC AND POLAROGRAPHIC STUDIES ON THE BLOOD OF ERYTHROBLASTOTIC BABIES

An electrophoretic and polarographic study of 25 erythroblastotic babies and their parents is here presented The conclusions are as follows

1 There is a great resemblance between the electrophoretic pattern of the proteins in both the father and child

2 The same resemblance in the behavior of father and child s proteins are also shown

Instituto Español de Hematología y Hemoterapia Madrid Spain

by polarography when in contact with denaturalizing agents such as pepsin and urea Both their proteins seem more labile when *compared to the* mother

3 The question is brought up whether this protein lability *is limited only* to the serum proteins or also to the erythrocytic globin *If this were true it would explain* all those cases that should have been erythroblastosis and were not and those which *in their t rn should not* have been according to the etiopathogenic theory for its production

VI communication 10

Further Observations on the Rh Antigen D[u]

BIRGER BROMAN*

At the last International Congress of Hematology in Cambridge in 1950 I read a short *paper about* Some Views on the Rh Antigen D In this I pointed out that the population in Sweden shows a very uneven distribution of the D antigen between C and E D was found in combination with E less often than was expected according to the idea that D arises as a mutation from D

Further investigations along the same lines have verified these first results 766 bloods *containing* either C or E though negative reacting with high titered agglutinating anti D test sera were examined as follows

	With only agglutinating serum	Antiglobulin pos	C e type
Assumed type Cde	526	164 CDe	Cde 362
Assumed type cdE	240	16 cDE	cdE 224
Ratio	2 2 1	10 1	1 6 1

These figures do not fit the mutation idea D is combined with C more often and with E less often than is expected According to the mutation theory this ratio should be 3 1 instead of the observed 10 1 The disturbance is statistically highly significant

We cannot at present give any explanation of these findings There seems to be some closer relationship between D and C than between D and E

Besides the genetic point of view the D antigen is of direct clinical interest As is known red cells containing the so called low grade D when suspended in saline are not at all agglutinated by anti D sera This D can be diagnosed after incubating the cells with blocking anti D serum and then performing an antiglobulin test Nevertheless the D can stimulate a D negative person to produce anti D and a D child born to an Rh negative women with D antibodies can be erythroblastotic

Most investigators therefore accepted the view that the D should be excluded when selecting Rh negative donors

However when a recipient or pregnant woman belongs to the D group there is some uncertainty as to whether it is possible to immunize her against the common antigen D or not It is often considered necessary to use Rh negative blood when giving transfusions to D In other words such patients are believed capable of producing anti D

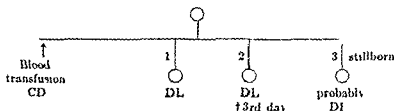
Our own clinical experience has shown that Rh immunized patients producing anti D never have D in their blood corpuscles even when they contain C or E This was also the

* Statens Rattskemiska Laboratorium Stockholm Sweden

finding of the late Dr Robert K Waller who had much experience of D in his work with Negroes in Virginia

Stronger support of the view that even as recipients and mothers persons with D ought to be considered Rh positive was derived from the following case

Cde/cde (after antiglobulin test the true type was found to be CD e/cde)



A woman with no D or E in her blood is repeatedly stimulated with D and E through transfusion and pregnancies She nevertheless produces only anti E (agglutinating titer 1:512)

Here we see a mother tested with agglutinating Rh typing sera who was found to be belong to the group Cde/cde (rh rh) Before her first pregnancy she was given one transfusion with Rh positive blood of the type CDe (Rh) After two pregnancies the children were found each to have inherited a cDE gene (R) from their father The second child died from erythroblastosis fetalis on the third day of life

The woman was found to be isoimmunized nevertheless she had produced only anti E (anti rh) No anti D (anti Rh_0) of any kind could be detected in her serum

Her third pregnancy ended in a macerated fetus and her anti E titer rose during this pregnancy In all likelihood also this fetus had inherited a cDE (R_2) gene but the mother in spite of this still produced only anti E and no anti D

Repeated tests of this mother s blood with different blocking anti D using the indirect antiglobulin method established the existence of a low grade D factor in the red cells

This case shows that a person whose blood contains the factor D of a very low grade when repeatedly stimulated with D (Rh_0) and with E (rh) is incapable of producing any anti D (anti Rh_0) though an anti E (anti rh) is produced

On the basis of these practical observations and in agreement with Stratton and Renton it seems from a clinical point of view justified to look upon D even the low grade forms as Rh positive factors

Nuevas Observaciones sobre el Antígeno Rh D

Sobre la base de las observaciones prácticas mencionadas en este trabajo y en concordancia con Stratton y Renton parecería desde un punto de vista clínico justificable considerar al antígeno D aun en sus formas más débiles como un factor Rh positivo

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	With [illegible]ly aggl [illegible]ting [illegible]rum	Ant glob pos	G[illegible]e type
Assumed type Cde	5[illegible]6	164 CD e	Cde 367
Assumed type cdE	240	*16 cD E*	*cdE* 2[illegible]4
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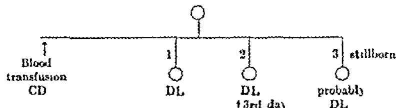
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NUEVAS OBSERVACIONES SOBRE EL ANTIGENO Rh D

Sobre la base de las observaciones prácticas mencionadas en este trabajo y en concordancia con Stratton y Renton parecería desde un punto de vista clínico justificable considerar al antígeno D aun en sus formas más debiles como un factor Rh positivo

VI communication 11

Erythroblastosis Due to Isoimmunization to the A B Factors

LUIS F SLPICH*

The erythroblastosis due to iso immunization of the mothers to the A B factors of th fetus is rare compared to the number cau ed by the Rh factor or if related to the total number of hetero specific pregnancies Of approximately 100 cases of hemolytic icterus of the newborn four or five belong to this group generally they are of benign course improving spontaneously and only exceptionally manifested by intense anemia

It is difficult to define which factors are responsible for the unleashing of the immunization process that arises from the fetal maternal incompatibility The role of the secretor character does not appear to be well defined of ten children born eight are secretors and two non secretors which is the proportion characteristic for the population in general the serious cases appear as much in one group as in the other

The constitutional factor added to the immunization conflict in some of the hetero specific pregnancies would explain a few of these cases as can be deduced from the study of some of the families included in this series

If Bernstein s theory of the anthropological significance of the A B O factors is admitted the hypothesis could be propounded that this type of immunization being produced in the first racial crossing and being repeated during thousands of generations has been the origin of the process of formation of the blood iso agglutinins alpha and beta which are found normally in the adult

Eritroblastosis por Inmunización a los Factores A B Síntesis de 9 Casos

La eritroblastosis que reconocen como origen la inmunización de las madres a los factores sanguíneos A B existentes en el feto son muy poco frecuentes si se las compara con las causadas por sensibilización al factor Rhesus o se las relaciona con el numero total de embarazos hetero específicos Aproximadamente de cada cien casos de ictericia hemolítica del recién nacido cuatro o cinco son de esta naturaleza en general son de curso benigno se restablecen espontáneamente y solo por excepción se manifiestan con intensa anemia

Es difícil señalar qué factores contribuyen a desencadenar el proceso inmunitario que nace de la incompatibilidad materno fetal el papel del carácter secretor no aparece bien definido de 10 niños enfermos 8 son secretores 2 no secretores proporcion semejante a la de esta característica en la poblacion en general los casos más serios se producen indistintamente en unos u otros

Un factor constitucional la fragilidad globular aumentada en el feto que se suma al conflicto inmunitario en algunos embarazos heteroespecíficos explicaría alguno de los casos segun se deduce del estudio de una de las familias incluidas en esta serie

Si se admite la teoría de Bernstein sobre el significado antropológico de los factores A B O puede esbozarse la hipotesis de que este tipo de inmunizacion al producirse en los primeros entrecruzamientos raciales y repetirse al través de millares de generaciones ha sido el origen del proceso de formacion de las iso aglutininas hemáticas alfa y beta que normalmente se hallan en el adulto

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La Prueba del Suero Antiglobulínico (Prueba de Coombs) en el Diagnóstico Diferencial de las Anemias Hemolíticas

MIGUEL A. ETCHEVERRY, EMILIO S. GUTIÉRREZ, ELMO L. CAPALBO y JORGE A. PEÑALVER*

Entre 301 pacientes estudiados la prueba de Coombs fué positiva en 105 y negativa en 196.

En estos 196 casos con prueba de Coombs negativa los antecedentes clínicos, serológicos y hematológicos confirmaron los resultados de la prueba de Coombs.

De los 105 casos con reacción positiva 85 correspondieron a recién nacidos con enfermedad hemolítica por incompatibilidad sanguínea feto materna en los cuales los antecedentes clínicos y hematológicos estuvieron de acuerdo con el resultado de la prueba de Coombs. En los 20 casos restantes con prueba positiva se trataba de anemias hemolíticas adquiridas de tipo inmunológico de las llamadas "por auto anticuerpos". Por el contrario en 14 casos de anemias hemolíticas congénitas esferocíticas y en los restantes casos de anemia hemolítica observados la prueba de Coombs fué constantemente negativa.

Los resultados obtenidos por nosotros con la prueba de Coombs confirman pues el valor de esta sencilla e ingeniosa prueba en el diagnóstico de la enfermedad hemolítica neonatal y en el diagnóstico diferencial de las anemias hemolíticas adquiridas del niño y del adulto.

The Coombs Antiglobulin Test in the Differential Diagnosis of Hemolytic Anemias

On 301 patients studied the Coombs test was positive in 105 and negative in 196. In these 196 cases with negative Coombs test the clinical, serological and hematological findings confirmed the negative results.

Of the 105 cases with positive results 85 corresponded to new born babies with hemolytic disease due to fetal maternal blood incompatibility. The clinical and hematological findings in these patients were in accord with the result of the Coombs test. In the other 20 positive cases the anemias were of the acquired hemolytic type also referred to as due to autoantibodies. On the other hand the 14 cases of spherocytic congenital hemolytic anemias showed a repeatedly negative Coombs. The rest of the anemias studied also were negative.

The results we have obtained by the use of the Coombs test confirm the value of this simple and ingenious test in the diagnosis of the neonatal hemolytic disease and the differential diagnosis of the acquired hemolytic anemia of the child and the adult.

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Erythroblastosis Due to Isoimmunization to the A B Factors

LUIS F SLPICH*

The erythroblastosis due to iso immunization of the mothers to the A B factors of the fetus is rare compared to the number caused by the Rh factor or if related to the total number of hetero specific pregnancies Of approximately 100 cases of hemolytic icterus of the newborn four or five belong to this group generally they are of benign course improving spontaneously and only exceptionally manifested by intense anemia

It is difficult to define which factors are responsible for the unleashing of the immunization process that arises from the fetal maternal incompatibility The role of the secretor character does not appear to be well defined of ten children born eight are secretors and two non secretors which is the proportion characteristic for the population in general the serious cases appear as much in one group as in the other

The constitutional factor added to the immunization conflict in some of the hetero specific pregnancies would explain a few of these cases as can be deduced from the study of some of the families included in this series

If Bernstein s theory of the anthropological significance of the A B O factors is admitted the hypothesis could be propounded that this type of immunization being produced in the first racial cro sing and being repeated during thousands of generations has been the origin of the process of formation of the blood iso agglutinins alpha and beta which are found normally in the adult

Eritroblastosis por Inmunización a los Factores A B Síntesis de 9 Casos

Las eritroblastosis que reconocen como origen la inmunización de las madres a los factores sanguíneos A B existentes en el feto son muy poco frecuentes si se las compara con las causadas por sensibilizacion al factor Rhesus o se las relaciona con el numero total de embarazos hetero específicos Aproximadamente de cada cien casos de ictericia hemolítica del recién nacido cuatro o cinco son de esta naturaleza en general son de curso benigno se restablecen espontáneamente y sólo por excepcion se manifiestan con intensa anemia

Es difícil señalar qué factores contribuyen a desencadenar el proceso inmunitario que nace de la incompatibilidad materno fetal el papel del carácter secretor no aparece bien definido de 10 niños enfermos 8 son secretores 2 no secretores proporcion semejante a la de esta característica en la poblacion en general los casos más serios se producen indistintamente en unos u otros

Un factor constitucional la fragilidad globular aumentada en el feto que se suma al conflicto inmunitario en algunos embarazos heteroespecíficos explicaría alguno de los casos segun se deduce del estudio de una de las familias incluídas en esta serie

Si se admite la teoría de Bernstein sobre el significado antropológico de los factores A B O puede esbozarse la hipotesis de que este tipo de inmunizacion al producirse en los primeros entrecruzamientos raciales y repetirse al través de millares de generaciones ha sido el origen del proceso de formacion de las iso aglutininas hemáticas alfa y beta que normalmente se hallan en el adulto

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disease In each the bone marrow was intensely hyperplastic which indicated that the stimulus of moderate anemia had evoked a maximal or nearly maximal erythropoietic response We measured the red cell volume in each patient and found it to be approximately 70 per cent of normal The total amount of circulating hemoglobin was about 7 grams per kg of body weight The average life span of our patients red cells in each case was found to be about 12 days This was established by transfusing the blood of a patient into a normal recipient Elimination of the transfused red cells was followed by the Ashby technic of differential agglutination The average survival time was determined by extending the rapidly falling component of the Ashby curve This met the base line at a point representing the average life span of the transfused cells

The rate of hemoglobin production in the four patients was computed in the same fashion as the normal The size of the mass of circulating hemoglobin was 7 grams per kg of body weight Life span of the hemoglobin averaged 12 days Therefore the rate of production equalled the mass divided by 12 or 600 mg per kg per day This is 7 times the normal rate of hemoglobin production

It is suggested that this figure reflects the limit of the ability of the human bone marrow to synthesize hemoglobin The actual capacity may be somewhat greater but this represents the order of magnitude of the maximal erythropoietic response

What does this mean in clinical terms? In the first place it demonstrates why these patients were anemic Their red cells survived only 1/10 the normal life span To compensate for such a short life span the marrow must produce 10 times the normal amount of hemoglobin It actually produced 7 times the normal or 70 per cent of the requirement As a consequence of this the mass of circulating hemoglobin in these patients was 70 per cent of normal This was the measure of their anemia

There is another clinical aspect to be considered If the average life span of these patients red cells had been 20 days instead of 12 there would have been no anemia A bone marrow that produces 7 times the normal amount of hemoglobin cannot compensate for destruction at 10 times the normal rate but it can compensate for 7 times the normal It can compensate for an average red cell life span that is 1/7 of 120 days or 17 days The average life span represented by this figure bears a critical relationship to the pathogenesis of hemolytic anemia This can be stated as a rule of thumb where the marrow is capable of a maximal response anemia exists if the average life span of the red cells is less than 15–20 days There is no anemia if the average life span is greater than 15–20 days This rule was derived theoretically In practice it seems to work rather well Red cells from some patients with hereditary spherocytosis have an average life span of 25 days These patients are not anemic In other patients with acquired hemolytic disease red cells survive less than 5 days These patients are severely anemic This suggests another rule of thumb the severity of anemia in hemolytic disease is a function of the average life span of the red cells provided of course there is a maximal effort to produce hemoglobin According to this rule there would be no anemia where the average red cell life span is 20 days or more There would be little anemia with a life span of 15 days moderate anemia at 10 days and severe anemia at 5 days These clinical rules of thumb cannot be applied in pernicious anemia Cooley s anemia leukemia and other conditions where a disease of the bone marrow itself may exist in addition to an abnormal hemolytic process

In summary hemolytic disease exists where the average life span of red cells is less than normal

Hemolytic anemia occurs when the maximal erythropoietic effort of the bone marrow is insufficient to compensate for the abnormally short life span of the circulating red cells

The maximal erythropoietic capacity of the human bone marrow as exemplified by its reaction in chronic hereditary hemolytic disease is about 7 times the normal

Under these conditions hemolytic anemia occurs when the average life span of the circulating red cells is less than 15–20 days The severity of hemolytic anemia is proportional to the shortness of the life span of the red cells

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The Rate of Hemoglobin Production in Chronic Hemolytic Anemia

WILLIAM H CROSBY and JOSEPH H AKEROYD*

An accurate definition of hemolytic disease was not possible until about 10 years ago Prior to that time we spoke of increased blood destruction but the phrase was indefinite because the normal rate of hemolysis was not known This deficiency of knowledge did not prevent our identifying important clinical states that exist as a consequence of abnormal hemolysis The clinical picture of hemolytic anemia depends upon a two fold reaction increased destruction of red blood cells and increased production of red cells The classical signs of increased destruction are acholuric jaundice increased excretion of bile pigments in the feces and splenomegaly The cardinal signs of increased production of red cells are erythroid hyperplasia of the bone marrow and the presence of abnormal numbers of reticulocytes in the peripheral blood Taken together these signs that comprise the hemolytic syndrome indicate the presence of rather severe hemolytic activity Taken separately none of them provides a definition of hemolytic disease Indeed a mild or moderate degree of hemolysis may exist without there being demonstrable any single one of the signs of the hemolytic syndrome

A definition of hemolytic disease became possible when the life span of normal human red cells was established It then became apparent that hemolytic disease was present where the average intravascular life span of the circulating red cells was less than normal Today it is generally accepted that the average normal life span of human red cells is between 100 and 120 days Therefore we may define hemolytic disease as any disease that results in an average red cell life span of less than 100 days

In proposing this definition it is recognized that there exist examples of hemolytic disease which have no clinical significance There are people whose red cells survive only 80 days or even 60 days yet they have no jaundice no reticulocytosis and no anemia The hemolytic disease—which is present according to the definition—is completely compensated The bone marrow produces enough extra red cells to make up for their abnormally short life span Increased erythropoiesis is the compensatory mechanism that prevents anemia in moderate hemolytic disease This brings us to the heart of our problem Why is there ever anemia in hemolytic disease? If there were no limit to hemoglobin production there would be no anemia even in severe hemolytic disease Hemolytic anemia occurs when the survival time of the red cells is so extremely short that the bone marrow is unable to increase its output sufficiently to compensate At this point and beyond the bone marrow achieves its maximum erythropoietic effort What is the magnitude of this effort in terms of normal production of hemoglobin? Is it twice the normal or ten times or fifty times? We have endeavored to learn this

The normal rate of hemoglobin production was easily computed from two well established facts The total mass of circulating hemoglobin in a normal man is about 10 grams per kg of body weight The life span of the hemoglobin is 120 days Each day 1/120 of the total mass of hemoglobin is destroyed and replaced The normal daily production is therefore 90 mg of hemoglobin per kg of body weight

The maximal rate of hemoglobin production in chronic hemolytic disease was studied in four well nourished healthy soldiers who had hereditary hemolytic anemia Two of them had hereditary spherocytosis the other two had hereditary nonspherocytic hemolytic

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disease In each the bone marrow was intensely hyperplastic which indicated that the stimulus of moderate anemia had evoked a maximal or nearly maximal erythropoietic response We measured the red cell volume in each patient and found it to be approximately 70 per cent of normal The total amount of circulating hemoglobin was about 7 grams per kg of body weight The average life span of our patients red cells in each case was found to be about 12 days This was established by transfusing the blood of a patient into a normal recipient Elimination of the transfused red cells was followed by the Ashby technic of differential agglutination The average survival time was determined by extending the rapidly falling component of the Ashby curve This met the base line at a point representing the average life span of the transfused cells

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La Capacidad de la Médula Osea para Producir Hemoglobina Su Relación con el Diagnóstico de las Enfermedades Hemolíticas

La medula osea humana normal produce alrededor de 0 09 g de Hb por kg de peso corporal y por día Investigaciones sugieren que la medula ósea en la anemia hemolitica hereditaria puede producir alrededor de 0 6 g por kg y por día lo que representa alrededor de siete veces la produccion normal de la eritropoyesis Por esto la médula es capaz de compensar un cierto volumen de hemolisis anormal

Teoricamente la anemia no debiera presentarse mientras la vida media de los globulos rojos no fuera menor que un séptimo de lo normal Si el término medio normal de supervivencia es alrededor de 120 días se deduce que la anemia debe presentarse cuando el término medio de vida de los globulos rojos sea menor de 1/7 de 120 días o sea alrededor de 18 días

Desde un punto de vista diagnostico se debe tener presente que la enfermedad hemolítica puede existir sin anemia cuando la médula osea produce suficiente hemoglobina para mantener un nivel normal de sangre circulante La anemia se produce cuando la supervivencia del glóbulo rojo es tan corta que el esfuerzo eritropoyético de la medula osea no es suficiente para reemplazarla El grado de anemia desarrollado de acuerdo al esfuerzo máximo de respuesta de la médula osea es funcion de la vida de los glóbulos rojos Se ha demostrado que pacientes cuyos globulos rojos tienen un promedio de vida de 25 dias no tienen anemia Pacientes con globulos rojos de promedio de vida de 12 días tienen anemia moderada Aquellos cuya supervivencia es de 5 días o menos tienen anemias severas

Cuando hay una inhibición de la eritropoyesis agregada a una hemólisis anormal la anemia puede ocurrir con globulos rojos de 25 días o más de supervivencia Esto ha sido demostrado en la anemia perniciosa moderada y en la anemia Mediterránea La producción de hemoglobina en estas enfermedades es de 0 25–0,35 g por kg de peso corporal y por día

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Fluid Absorption by Red Blood Cells and Hemolysis in Experimental Venous Stasis

G ROSENFELD S SCHENBERG and L NAHAS*

Absorption of fluid by the red blood cells causing increase in their volume a decrease of globular resistance and hemolysis was reported by Rosenfeld in shock induced in dogs by histamine trypsin peptone and bothropic venom injections As these substances except the bothropic venom did not produce any change of red blood cells in vitro this author considered these modifications as due to shock provoked by the substance and not directly due to the drug

Rosenfeld Nahas Leao Beraldo and Schenberg observed later the same phenomenon in tourniquet induced shock in dogs As the alterations were the same even in absence of arterial pressure fall investigations were made in order to verify if they are chiefly due to venous stasis in a determined region and which are their relations with the intensity of arterial pressure fall

Dogs were anesthetized with nembutal by intraperitoneal route and the arterial pressure was graphically recorded The mesenteric vein was clamped for variable periods of time The red blood cells were counted and hematocrit quantitative globular resistance total and free hemoglobin in plasma and hemosedimentation were determined Control

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experiments for the changes caused by anesthesia with and without laparotomy and handling of the abdominal viscerae were also made

There was observed an increase of the mean corpuscular volume hemolysis and decrease of globular resistance hemoconcentration and decrease of sedimentation rate of the red blood cells caused by clamping of the mesenteric vein Clamping of the vena cava or handling of the abdominal viscerae caused alterations of the same type but less marked These changes appeared even when there was no appreciable fall of the arterial pressure

ABSORCION DE LIQUIDO POR GLOBULOS ROJOS Y HEMOLISIS EXPERIMENTAL EN ESTASIS VENOSAS

La absorción de líquidos por glóbulos rojos causando aumento de su volumen disminución de resistencia globular y hemólisis fué relatada por Rosenfeld en shock inducido en perros con histamina tripsina peptona e inyecciones de veneno bothrópico Como estas substancias menos el veneno bothrópico no produjeron ningun cambio en los glóbulos rojos in vitro este autor consideró que estas modificaciones se debían al shock provocado por la substancia y no directamente a la droga

Rosenfeld Nahas Leão Beraldo y Schenberg observaron posteriormente el mismo fenómeno en shock provocado por torniquete en perros Como las alteraciones eran las mismas aun faltando la caída de la presión arterial se hicieron investigaciones con el objeto de verificar si éstas se deben principalmente a estasis venosas en determinada región y cuáles son sus relaciones con la intensidad de la caída de la presión arterial

Se anestesiaron perros con nembutal por vía intraperitoneal y la presión arterial fué tomada gráficamente La vena mesentérica fué comprimida por distintos períodos de tiempo Se contaron los glóbulos rojos y el hematocrito y fueron determinadas la resistencia globular cuantitativa la hemoglobina total y libre en el plasma y la hemosedimentación

También se hicieron experimentos para controlar los cambios provocados por anestesia con o sin laparatomía y manejo de las vísceras abdominales Se observó un aumento del volumen corpuscular medio hemólisis y disminución de la resistencia globular hemoconcentración y disminución del grado de sedimentación de los glóbulos rojos provocados por la compresión de la vena mesentérica

La compresión de la vena cava o el manejo de las vísceras abdominales causaron alteraciones del mismo tipo pero menos marcadas Estos cambios aparecieron aun cuando no había una apreciable caída de la presión arterial

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Absorption of Fluid by Red Blood Cells and Hemolysis in Shock Induced by Tourniquet

G ROSENFELD S SCHENBERG and L NAHAS*

The increase of red blood cell volume as a result of fluid absorption by them decrease of globular resistance and hemolysis were reported by Rosenfeld in shock induced in dogs by trypsin peptone bothropic snake venom and histamine injection According to this author these hematologic changes are not caused directly by the injected drugs but by the shock provoked by these substances Coonse et al inducing traumatic and hemorrhagic shock in dogs noticed hemolysis in the traumatic shock

In order to verify the occurrence of these modifications in shocks of different nature tourniquet shock was induced in dogs

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In dogs anesthetized with nembutal by intraperitoneal route the tourniquet has been fitted in one hind leg in two groups of animals in the first the tourniquet remained for about 6 hours in another one for 12 to 19 hours Red blood cell and leukocyte counts were made and total hemoglobin hemosedimentation rate hematocrit and quantitative globular resistance were determined

The first hematologic examination was made before the tourniquet was fitted and another one was made just before the tourniquet was removed Further blood samples were taken at variable time intervals after removing the tourniquet

The results obtained depicted increase in the mean corpuscular volume hemoconcentration decreases of hemosedimentation rate decrease of globular resistance hemolysis and neutrophilic leukocytosis All these modifications were present even before the tourniquet was removed and they took place even without blood pressure fall The latter was not frequent in all dogs Some dogs showed an intensification of the stage already attained after tourniquet removal The group that remained with the tourniquet for more than 12 hours did not present greater intensity of hematologic changes

Absorción de Líquido por Glóbulos Rojos y Hemolisis en el Shock Provocado por Torniquete

El aumento del volumen de los glóbulos rojos como resultado de la absorcion de líquido por ellos la disminucion de la resistencia globular y hemolisis fueron estudiados por Rosenfeld en shocks provocados en perros por la tripsina peptona veneno bothropico de los ofidios e inyecciones de histamina De acuerdo a este autor estos cambios hematologicos no son provocados directamente por las drogas inyectadas sino por el shock provocado por estas substancias Coonse et al provocando shocks traumáticos y hemorrágicos en perros notaron hemolisis en los shocks traumáticos

Para verificar las modificaciones de distinta naturaleza en los shocks se provocaron en los perros shocks por torniquete

En perros anestesiados con nembutal por vía intraperitoneal el torniquete se fijo en una pata trasera en dos grupos de animales En el primero el torniquete se dejó durante 6 horas en el otro de 12 a 19 horas Se hizo el recuento de globulos rojos y leucocitos y se determinó la hemoglobina total la eritrosedimentación el hematocrito y la resistencia globular cuantitativa

El primer examen hematologico se hizo antes de aplicarse el torniquete y luego otro se hizo justo antes de ser retirado Otros recuentos se hicieron en tiempos variados después de quitarse el torniquete

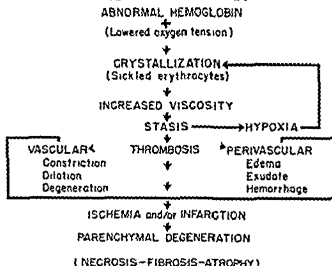
Los resultados demostraron aumento en el volumen corpuscular medio hemoconcentracion disminución de la velocidad de la eritrosedimentación disminucion de la resistencia globular hemólisis y leucocitosis neutrofilica Estas modificaciones estaban presentes antes de ser retirado el torniquete y se produjeron sin que cayera la presion sanguinea Esto ultimo no sucedio con todos los perros En algunos se observó una intensificacion del estado alcanzado después de quitarles el torniquete El grupo de perros que permanecio con el torniquete por más de 12 horas no presentaba mayor intensidad en los cambios hematologicos

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Sickle Cell Anemia, a Vascular Occlusive Disease

COLIN F VORDER BRUEGGE and L W DIGGS*

The abnormal hemoglobin in individuals with the sickle cell trait crystallizes on deoxygenation. Crystallization of reduced hemoglobin within erythrocytes produces relatively rigid brittle elongate and spiculate forms which pass with more difficulty through narrowed spaces than do normal cells. It has been demonstrated that there is a significant increase in the viscosity of blood in which the erythrocytes are sickled as compared with oxygenated blood in which the cells are of normal shape. Increased viscosity of the blood interferes with blood flow and tends to produce capillary blockade, vascular stasis and sequestration of red cells in the spleen and other viscera. Stasis of blood causes hypoxia and hypoxia in turn causes an exaggeration of the sickling process (see the diagram).



Physiological and anatomical changes resulting from stasis and hypoxia include

Vasospasm and vasodilation	
Increased plasma permeability	perivascular edema
Perivascular cellular infiltration	
Perivascular hemorrhage	
Degenerative changes in vessel walls	
Hemoconcentration	
Thrombosis	embolism infarction
Injury to erythrocytes causing	
Increased hemolysis	
Increased mechanical fragility	Anemia
Increased likelihood of phagocytosis	Hemolytic jaundice

Other factors which play a role in the production of obliterative vascular phenomena are

1) Fat emboli in occasional patients due to occlusive vascular processes in fatty bone

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marrow with the production of fat necrosis and the liberation of fat droplets into the systemic circulation (Vance and Fisher Wade and Stevenson Wertham et al and Wyatt and Orrahood)

2) Narrowing of the lumen of blood vessels due to intimal and subintimal proliferative changes

In some patients with sickle cell anemia the occlusive vascular changes take place slowly over a period of years causing a gradual replacement of parenchyma by fibrous tissue and progressive reduction in the volume of the vascular bed These changes resemble the normal ageing process but take place at an accelerated rate In other patients there may be massive tissue destruction due to sudden occlusion of larger blood vessels often causing death in early years or disability at any age Local ischemia in various viscera due to the vascular occlusion is thought to be the explanation for the painful and febrile episodes or crisis Hemolysis of blood and anemia are associated phenomena but are thought to play a relatively minor role in the socalled crisis and to be secondary rather than primary factors The obliterative vascular lesions in sickle cell anemia with progressive fibrous tissue replacement of parenchyma are consistently present in the spleen but similar evidence of vascular occlusion necrosis atrophy and fibrosis may be demonstrable in any organ of the body

La Anemia Falciforme una Enfermedad Vascular Oclusiva

La hemoglobina anormal de los individuos con anemia falciforme sufre una cristalizacion cuando hay reduccion de oxigeno Esto da lugar a eritrocitos caracterizados por tener formas elongadas o en espicula relativamente rígidas y frágiles que pasan con mayor dificultad que los hematies normales a través de espacios estrechos Se ha demonstrado también un aumento de la viscosidad sanguínea que tiende a producir bloqueos capilares estasis vascular y secuestro de hematíes en el bazo y otros organos La estasis de la sangre causa hipoxia y la hipoxia a su vez causa una exageración de las alteraciones eritrocíticas

Los cambios anatomicos y fisiologicos resultantes de la estasis y de la hipoxia incluyen

Vaso espasmo y vasodilatacion
Aumento de la permeabilidad plasmática — edema perivascular
Infiltracion celular perivascular
Hemorragia perivascular
Cambios degenerativos en las paredes de los vasos

Hemoconcentracion
Trombosis — embolias infarto
Lesiones a los eritrocitos causando
Aumento de la hemolisis
Aumento de la fragilidad mecánica } Anemia
Mayor suceptibilidad a la fagocitosis } Ictericia Hemolitica

Otros factores de importancia en la produccion de los fenómenos vasculares oclusivos son

1) Embolias de grasa en algunos casos debidas a procesos vasculares oclusivos de la medula osea grasa con produccion de necrosis lipoidea y liberacion de pequeñas gotas de grasa a la circulacion

2) Estrechamiento del lumen de los vasos debido a cambios proliferativos de la intima y subintima

En algunos pacientes con anemia falciforme los cambios vasculares oclusivos se producen en forma lenta y sobre un periodo de anos ocasionando un reemplazo gradual de tejido parenquimatoso por tejido fibroso y una reduccion gradual en el volumen del lecho vascular En otros pacientes puede haber destruccion tisular masiva debido a una oclusion subita de vasos mayores que pueden ocasionar la muerte o invalidez del paciente La isquemia local producida por oclusion vascular en distintos organos puede ser la causa de

los episodios febriles y dolorosos *Las lesiones vasculares oclusivas de la anemia falciforme* con remplazo progresivo del tejido parenquimatoso por tejido fibroso se encuentran siempre presentes en el bazo pero evidencias similares de oclusion vascular necrosis atrofia y fibrosis pueden ser halladas en cualquiera de los demás organos

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Influence of ACTH and Cortisone on Experimental Antibody Production

S MOESCHLIN and R BAGUENA*

In rabbits sensitized with typhus vaccine a marked rise of the agglutinin titer in the blood (appearing on the 5th and 6th days) is produced by reinjection of the vaccine If high doses of ACTH or cortisone are administered to these animals from the moment of reinjection no decrease in the production of antibodies is observed Thus the ACTH or cortisone has no direct inhibitory effect on antibody formation In addition the treatment with these hormones *beginning two hours before the reinjection does not change* the production of the immune serum which is further evidence that ACTH and cortisone do not exert a primary inhibitory effect on antibody production However when treatment with the hormone is begun one week or longer prior to the vaccine reinjection a very pronounced inhibition of antibody production is effected From this alteration in effect it is postulated that *ACTH and cortisone do not inhibit the actual immune body formation* but instead the absorption and splitting of the bacteria into the specific antigens (protein products) Thus antibody production is probably not primarily inhibited by ACTH and cortisone but rather suffers from the secondary effect of slowing of antigen absorption and break down

Influencia del ACTH y de la Cortisona sobre la Producción Experimental de Anticuerpos

En conejos previamente sometidos a una vacunación antitífica la reinyección intravenosa de esa vacuna produce después de 5 a 6 días un ascenso acentuado del título de *aglutininas en la sangre Si se administran a estos animales dosis elevadas de ACTH o de* Cortisona desde el momento de la reinyección de la vacuna no se observa disminución en la producción de los anticuerpos Por lo tanto el ACTH o Cortisona no tienen efecto inhibidor directo sobre la formación de anticuerpos Lo mismo ocurre si se administran estas hormonas dos horas antes de la reinyección vaccinal Se concluye pues que la Cortisona y el ACTH no tienen acción inhibidora directa sobre la formación de anticuerpos Si por el contrario se comienza la administración de las hormonas una semana o más antes de la *reinyección ocurre una neta inhibición de la formación de los anticuerpos Se puede* concluir pues que el ACTH y la Cortisona no inhiben la formación de los anticuerpos en el sentido estricto de la palabra sino más bien inhibe la reabsorción y la desintegración de los micro organismos en antígenos específicos (fracciones proteicas) Es probable pues que el ACTH y la Cortisona no tengan una acción inhibidora primaria sobre la formación de la inmunidad sino una acción secundaria por freno de la reabsorción y fraccionamiento de los antígenos

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marrow with the production of fat necrosis and the liberation of fat droplets into the systemic circulation (Vance and Fisher Wade and Stevenson Wertham et al and Wyatt and Orrahood)

2) Narrowing of the lumen of blood vessels due to intimal and subintimal proliferative changes

In some patients with sickle cell anemia the occlusive vascular changes take place slowly over a period of years causing a gradual replacement of parenchyma by fibrous tissue and progressive reduction in the volume of the vascular bed These changes resemble the normal ageing process but take place at an accelerated rate In other patients there may be massive tissue destruction due to sudden occlusion of larger blood vessels often causing death in early years or disability at any age Local ischemia in various viscera due to the vascular occlusion is thought to be the explanation for the painful and febrile episodes or crisis Hemolysis of blood and anemia are associated phenomena but are thought to play a relatively minor role in the socalled crisis and to be secondary rather than primary factors *The obliterative vascular lesions in sickle cell* anemia with progressive fibrous tissue replacement of parenchyma are consistently present in the spleen but similar evidence of vascular occlusion necrosis atrophy and fibrosis may be demonstrable in any *organ of the body*

La Anemia Falciforme una Enfermedad Vascular Oclusiva

La hemoglobina anormal de los individuos con anemia falciforme sufre una cristalización cuando hay reduccion de oxígeno Esto da lugar a eritrocitos caracterizados por tener formas elongadas o en espícula relativamente rígidas y frágiles que pasan con mayor dificultad que los hematíes normales a través de espacios estrechos Se ha demonstrado también un aumento de la viscosidad sanguínea que tiende a producir bloqueos capilares estasis vascular y secuestro de hematíes en el bazo y otros organos La estasis de la sangre causa hipoxia y la hipoxia a su vez causa una exageración de las alteraciones eritrocíticas

Los cambios anatomicos y fisiologicos resultantes de la estasis y de la hipoxia incluyen
Vaso espasmo y vasodilatacion
Aumento de la permeabilidad plasmática — edema perivascular
Infiltracion celular perivascular
Hemorragia perivascular
Cambios degenerativos en las paredes de los vasos

Hemoconcentracion
Trombosis — embolias infarto
Lesiones a los eritrocitos causando
Aumento de la hemolisis
Aumento de la fragilidad mecánica } {Anemia
Mayor susceptibilidad a la fagocitosis } {Ictericia Hemolitica

Otros factores de importancia en la produccion de los fenomenos vasculares oclusivos son

1) Embolias de grasa en algunos casos debidas a procesos vasculares oclusivos de la medula osea grasa con produccion de necrosis lipoidea y liberacion de pequenas gotas de grasa a la circulacion

2) Estrechamiento del lumen de los vasos debido a cambios proliferativos de la íntima y subintima

En algunos pacientes con anemia falciforme los cambios vasculares oclusivos se producen en forma lenta y sobre un período de anos ocasionando un reemplazo gradual de tejido parenquimato o por tejido fibroso y una reduccion gradual en el volumen del lecho vascular En otros pacientes puede haber destruccion tisular masiva debido a una oclusion subita de vasos mayores que pueden ocasionar la muerte o invalidez del paciente La isquemia local producida por oclusion vascular en distintos organos puede ser la causa de

Los episodios febriles y dolorosos. Las lesiones vasculares oclusivas de la anemia falciforme con reemplazo progresivo del tejido parenquimatoso por tejido fibroso se encuentran siempre presentes en el bazo pero evidencias similares de oclusión vascular, necrosis, atrofia y fibrosis pueden ser halladas en cualquiera de los demás órganos.

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INFLUENCIA DEL ACTH Y DE LA CORTISONA SOBRE LA PRODUCCIÓN EXPERIMENTAL DE ANTICUERPOS

En conejos previamente sometidos a una vacunación antitífica la reinyección intravenosa de esa vacuna produce después de 5 a 6 días un ascenso acentuado del título de aglutininas en la sangre. Si se administra a estos animales dosis elevadas de ACTH o de Cortisona desde el momento de la reinyección de la vacuna no se observa disminución en la producción de los anticuerpos. Por lo tanto el ACTH y Cortisona no tienen efecto inhibidor directo sobre la formación de anticuerpos. Lo mismo ocurre si se administran estas hormonas 2 horas antes de la reinyección vaccinal. Se concluye pues que la Cortisona y el ACTH no tienen acción inhibidora directa sobre la formación de anticuerpos. Si por el contrario se comienza la administración de las hormonas una semana o más antes de la reinyección ocurre una neta inhibición de la formación de los anticuerpos. Se puede concluir pues que el ACTH y la Cortisona no inhiben la formación de los anticuerpos en el sentido estricto de la palabra sino más bien inhiben la reabsorción y la desintegración de los microorganismos en antígenos específicos (fracciones proteicas). Lo probable pues que el ACTH y la Cortisona no tengan una acción inhibidora primaria sobre la formación de la inmunidad sino una acción secundaria por freno de la reabsorción y fraccionamiento de los antígenos.

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The Relationship between Blood Groups and Susceptibility to Hemolytic Anemia

S P LUCIA and M L HUNT*

In a study of 46 cases of hemolytic anemia (22 congenital 23 acquired and one of indeterminate type) it was observed that subjects of blood group O appeared to be more vulnerable to the hemolytic process The frequency of the blood groups in the cases under study were group O 65.2% group A 19.6% group B 5.7% and group AB no subjects encountered A further analysis of the data revealed that in 23 cases of *acquired* hemolytic anemia 19 were of group O an incidence of 82.7% The mechanism of hemolytic anemias is discussed in relation to certain phenomena of immunization

La Relación entre los Grupos Sanguíneos y Susceptibilidad a la Anemia Hemolítica

En un estudio de 46 casos de anemia hemolitica (22 congénitas 23 adquiridas y 1 de tipo indeterminado) se observo que los sujetos pertenecientes al grupo O parecian ser más vulnerables a los procesos hemoliticos La frecuencia de los grupos sanguíneos en los casos sometidos a estudio era Grupo O 65.2% grupo A 19.6% grupo B 5.7% y grupo AB ninguno Un análisis ulterior de los datos revelo que en 23 casos de anemia hemolitica adquirida 19 eran de grupo O con una incidencia de 82.7% Se discute el mecanismo de las anemias hemolíticas en relacion con ciertos fenomenos de inmunizacion

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A Case of Nocturnal Hemoglobinuric Hemolytic Anemia

D CIOCIA†

The writer reports one case studied of nocturnal paroxysmal hemoglobinuric hemolytic anemia without spleen tumor with normal resistance of the red cells to osmotic solutions (Marchiafava Micheli) All therapeutic measures practiced so far have failed The pathogenesis of this disease has not been solved and the authors have given pathogenic importance to the blood pH primarily altered to the hemoglobin alteration and to a perturbed function of the spleen or of the reticulo endothelial system Studies of the hemolysis of the diseased blood in vitro have shown a hemolizing capacity of its serum even for the red cells of a healthy individual of the same blood group

The author has obtained a complete remission of the hemolytic hemoglobinuric crisis by treating the patient repeatedly with normal human serum

With this treatment the serious ly ill patient got better even by leaving off all other anti anemic therapeutic measures and was able to start work again providing he underwent a periodic treatment with normal human serum

The therapeutic effect of human serum in this disorder has been proved in some other blood diseases in which it seems to act also by giving the sick body a factor that normalizes the protein and enzymatic properties of the serum

Fatebenefratelli Hospital Milan Italy

Anemia Hemolítica Hemoglobinúrica Paroxística

El autor describe un caso llegado a su observación de anemia hemolítica hemoglobinúrica paroxística nocturna, sin tumor de bazo, con resistencia osmótica normal de los glóbulos rojos, llamada por la escuela italiana con el nombre de Marchiafava y Micheli, quienes la describieron primeramente, diferenciándola de la ictero anemia hemolítica constitucional. Todas las terapéuticas de esta enfermedad ensayadas hasta la fecha han fracasado. La patogenia misma no ha sido resuelta y los autores han dado importancia patogénica al pH de la sangre alterado primariamente, a la alteración de la hemoglobina y a una función perturbada del bazo o del sistema retículo endotelial.

El estudio de la hemolisis in vitro de la sangre enferma ha demostrado una capacidad hemolizante del suero de la sangre enferma aún sobre los hematíes de un individuo sano perteneciente al mismo grupo sanguíneo. El autor ha obtenido una remisión de las crisis hemolíticas hemoglobinúricas con la provisión repetida de suero humano normal. Con esta terapéutica el enfermo, destinado al desenlace fatal y ya con el mínimo de glóbulos rojos tolerable, pudo restablecerse, aún dejando de lado todas las otras terapéuticas antianémicas y pudo retomar su trabajo subordinándose a la provisión periódica de suero humano normal.

El efecto terapéutico del suero humano en esta enfermedad ha sido confirmado en otras enfermedades de la sangre, en las cuales también parece actuar aportando al organismo enfermo un quid que normaliza las propiedades proteicas y enzimáticas del suero.

PART VII

Hemorrhagic Disturbances

Trastornos Hemorrágicos

Nuestra Experiencia en la Hemofilia (Consideraciones Clínicas, Patogénicas, Diagnósticas y Terapéuticas)*

ALFREDO PAVLOVSKY, MARIANO R. CASTEX, CELIA SIMONETTI DE ARBONA, DAVID CORONADO, LUCIA DUMAS, HORACIO CASTELLANOS, DORA MITTELMAN, EDUARDO PEDACE, ROBERTO PATERSON TOLEDO, JUAN MENDIVE y ALBERTO ANDINO

CONSIDERACIONES ESTADÍSTICAS

PRESENTAMOS las características más sobresalientes de 55 hemofílicos asistidos en la Academia Nacional de Medicina.

Pertenecen a 49 familias; excepto 13 familias—26,53%—las 36 restantes no registraron antecedentes hereditarios hemorragíparos.

Sólo 12 familias tienen 2-3 hijos hemofílicos; las otras 37 tienen un hijo enfermo y los demás varones sanos en ocasiones 9.

Dentro del primer año de edad se observaron los primeros signos en un 65,5% y dentro de los 2 años de edad en un 85%, destacándose los casos cuyos primeros síntomas se presentaron tardíamente, en uno de ellos a los 23 años. El 50% de los enfermos iniciaron la enfermedad con hematomas, el 31% con hemorragias consecutivas a heridas del frenillo, circuncisión, sección del cordón umbilical, etc., y el 19% restante con epistaxis, hematurias, hemartrosis o gingivorragias.

De 473 accidentes hemorrágicos espontáneos tabulados el 40% correspondió a hemartrosis, 30% a hematomas, 9% hematurias y los restantes fueron epistaxis, gingivorragias, hemorragias cerebromeníngeas, enterorragias, etc.

Todos nuestros hemofílicos—100%—en algún momento de su enfermedad padecieron de hemartrosis.

Estudiando la distribución mensual de 544 episodios hemorrágicos espontáneos durante varios años se observó un significativo aumento durante los meses de primavera—Septiembre, Octubre y Noviembre—para declinar a mediados de Diciembre.

La gravedad y frecuencia de los accidentes fué más acentuada entre los 5 y 15 años de edad, declinando luego en la edad adulta y madurez.

La evolución de algunos de ellos ha podido ser seguida durante más de 20 años.

Hemos tenido que lamentar 7 muertes—12,7%—cuatro de ellos por hemorragia cerebromeníngea; los otros tres restantes no fueron atendidos por nosotros en el momento de las hemorragias.

Mencionamos algunas intervenciones quirúrgicas a que han sido sometidos nuestros enfermos: nefrectomía, apendicectomía, cerclaje de rótula y numerosísimas extracciones dentarias, todas con éxito.

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* Resumen de los trabajos realizados en la Sección de Hematología del Instituto de Investigaciones Físicas de la Academia Nacional de Medicina de Buenos Aires, Argentina.

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Acción Coagulante de la Fracción Globulina de Distintos Hemofílicos y Otras Afecciones sobre el Tiempo de Coagulación del Hemofílico 9

Normales	
1	5 min a 1 min
2	35 min a 2 min 40 s
3	35 min a 1 min 37 s
4	33 min a 1 min 23 s
5	45 min a 40 s
Hemofílicos	
9 (t c 45 min)	35 min a 10 min 56 s
2 (t c 90 min)	35 min a 1 min 43 s
13 (t c 60 min)	55 min a 26 min
1 (t c 100 min)	35 min a 3 min
1 (t c 120 min)	45 min a 1 min
5 (t c 57 min)	35 min a 20 min
8 (t c 16 min)	58 min a 1 min 31 s
Trombopenias	
1	35 min a 1 min 15 s
2	35 min a 2 min 58 s
Anemia Perniciosa	
1	38 min a 1 min 3 s
2	31 min a 1 min 15 s
3	31 min a 2 min

tiempo de coagulación de los hemofílicos a mayor tiempo de coagulación se necesita mayor cantidad de sangre uno de ellos H 1 con T C de 80 min necesitó 600 ml de plasma normal

Observaron la ventaja de la concentración del citrato de sodio 3 8% sobre la de 7% en la transfusión de grandes cantidades de plasma

En 1944 Pavlovsky y Simonetti estudiaron la actividad coagulante de la fracción globulina de Patek y Taylor extraída de plasmas hemofílicos La actividad no guardó relación con el tiempo de coagulación y fué poco valiosa con excepción de los hemofílicos 1 2 y 8 con tiempos de coagulación muy largos y actividad globulínica tan intensa como la extraída de plasmas normales

Cartes Pavlovsky y Simonetti comprobaron el hecho paradojal que ciertos plasmas hemofílicos 1 2 y 8 con tiempos de coagulación muy largos eran capaces de acortar el tiempo de coagulación de otros hemofílicos

Transfundieron plasma del H 1 con T C de 80 min al H 9 reduciéndole el tiempo de coagulación de 30 a 6 minutos

Observaron el aumento del poder coagulante de la sangre hemofílica por el estacionamiento este aumento de actividad siendo más notable en la sangre total que en el plasma

Posteriormente 1948 a 1951 Pavlovsky Castellanos y Mittelman confirmaron los trabajos anteriores referentes a la fracción globulina de los hemofílicos, ha

PLAQUETAS
(Tromboplastinogena a
d Qui k)
CO-FACTOR
PLASMA
E ima trombopla ti d F gus
T bocat li ina d Leggenhage
E ima cti d ra d la trombopla tina
plaqueta ia d Ma F lane
Tromb li ina de B inkhous
GLOBULINA ANTIHEMOFILICA
INACTIVA (H mofili os)
ACTIVA (A rmal Alg H mofili os)
TROMBOPLASTINOGENO
TROMBOPLASTINA
ALBUMINA PLASMATICA (P i aly)
ANTICEFALINA (Tocan ins)
INACTIVADORES

FIG 1 —Patogenia de la Hemofilia

El principal impedimento físico de estos enfermos ha sido las artropatías hemofílicas llevan la mayoría de ellos una vida activa señalando como casos excepcionales que dos de ellos juegan al foot ball

Patogenia

Sustentamos la creencia que en la patogenia de la hemofilia deben contemplarse dos factores el vascular y el sanguíneo

En cuanto al defecto vascular surge de la experiencia clínica y no experimental 1° por la relativa benignidad de la fibrinopenia congénita con relación a la hemofilia aun con aquellos enfermos de tiempo de coagulación corto 2 por los largos períodos de bienestar hasta dos años en nuestros casos sin crisis hemorrágicas sin modificación alguna del tiempo de coagulación 3 por la producción de hemorragias en hemofílicos a quienes hemos mantenido en tiempo de coagulación reducido con transfusiones efectuadas diariamente 4 por la detención de las hemorragias actuando solo sobre el factor vascular etc nos hablan bien a las claras de la participación de los vasos que pueden fragilizarse o alterar su permeabilidad a raíz de desequilibrios de naturaleza aun no conocida humoral? nerviosa?

Nuestro Concepto Patogenético sobre la Alteración de la Coagulación

Castex y Pavlovsky en 1941 estudiando el tiempo de coagulación del plasma recalcificado en los hemofílicos observaron un alargamiento en el plasma privado de plaquetas por centrifugación prolongada cuando la determinación se efectúa a los pocos minutos de la extracción Si se dejan estacionar ambos plasmas (con y sin plaquetas) observaron a veces un acortamiento espontáneo del tiempo de coagulación lo que induce a pensar que no puede deberse simplemente a la desintegración de las plaquetas

Castex y Pavlovsky en 1943 comprueban que en general para normalizar el

ciando e en el di tinto comportamiento frente a sustancias absorbentes verbi gracia el Soy Ba

No pudieron confirmar la accion anticoagulante de las acido albuminas de Fons y

La 1940 comprueban el desarrollo de un anticoagulante en el hemofilico 1 que se encuentra ligado a la fraccion globulina del plasma

Tratamiento

Recomendamos una vida higiénica notific icion física y moral Profilaxis de los traumatismos

Tratamientos Generales Eficaces para Combatir los Accidentes Hemorrágicos o Prepararlos para las Intervenciones Quirurgicas

Transfusion de sangre plasma fresco congelado o liofilizado, globulina anti hemofilica normal

Desechamos las transfusiones profilacticas por el peligro de crear estados refractarios

Localmente compresion de la herida ligadura de vasos y sutura cuidadosas Aplicación de tromboplastinas de leche humana placenta humana, de cerebro de conejo y trombina

Tratamiento ortopédico producida la hemartrosis inmovilización temporal de las articulaciones hasta el cese del dolor en la posicion que se encuentra la articulacion Traccion discontinua de partes blandas reeducacion articular masajes etc

Tratamiento odontoulgico Nos referimos a las extracciones dentarias Previa transfusion de sangre o plasma se extrae el diente luego limpieza muy cuidadosa de la loge dentaria, taponamiento profundo de la misma aplicando localmente tromboplastinas preferentemente polvo de placenta humana

Dos palabras sobre la preparacion de ésta la placenta humana fresca es privada de sus membranas picada finamente y desecada al vacío luego se pulveriza con un molinillo de mano y se conserva en ampollas cerrada al vacío

Our Experience in Hemophilia

Statistical Considerations

We present the outstanding features of 5o hemophiliacs treated in the National Academy of Medicine They belong to 49 families and except in 13 of these (2(53%) there was no family history of bleeders In the majority of the cases (37) there is a number of brothers who are healthy and do not bleed (in some instances as many as 9) The spontaneous hemorrhagic accidents were as follows hemarthrosis 40% hematomas 30% hematurias 9% the rest of the cases being bleeding episodes from nose gums bowels etc

The bleeding episodes were more frequent during the spring and the frequency and severity of these was found to be most between the ages of 5 and 15 years declining later in the adult age

Pathogenesis

We believe that in the pathogenesis of hemophilia two factors must be contemplated the vascular and the blood factor The vascular defect is shown from the clinical experi

Placenta Humana Preparación y Dosaje de la Actividad Coagulante

(1) Tomar una placenta sana (2) separar la pulpa de la membrana que la recubre (3) pasar la pulpa por la máquina de picar (4) extender el picado sobre un vidrio (5) colocarlo en el desecador al vacío a 50 durante 24 horas con cloruro de calcio en el estante inferior (6) retirar la placenta desecada y pasarla por el molinillo para obtener el polvo (7) envasarlo en ampollas cerrarlas al vacío

Comparacion de la Actividad Coagulante de

	red c T C de
Tromboplastina de conejo	47 a 1
Leche humana fresca	40 a 1 15
Leche humana en polvo	87 a 1 17
Crema de Leche humana tratada con acetona	47 a 48
Placenta humana	47 a 50

Estudio Comparativo del Poder Coagulante de los Distintos Componentes de la Placenta

	red e T C de
Placenta total fresca	42 a 1 40
Placenta total desecada	45 a 1 15
Placenta total desecada tratada con acetona	30 a 1 14
Membrana	45 a 2
Cordon	60 a 1 6
Globulina de placenta (técnica de Eley y Green Mac Kham)	25 a 2 5″

Conservacion de la Actividad Coagulante

	Place ta f e ca	G dad en h l dera	Gong lad
Preparada recientemente	42 a 1 40	60 a 1 30	42 a 1 50
34 dias			40 a 1 [illegible]
42 dias		42 a 2 40	
5 meses			50 a 2 5

Plac ta d cad	R e t p epa n	T empo d c n erva
Guardada al vacío	50 a 1 40	1 ano 40 a 2 50
Sin vacio		1 ano 43 a 14 15

(Dr A Pavlovsky C S de Arbona L Dumas Acción coagulante de la placenta humana El Día Médico 1947)

ciendo notar que no solo los hemofilicos 1 2 y 8 corrigen el tiempo de coagulacion de los otros hemofilicos sino también el consumo de protrombina

La mezcla de plasma de hemofilicos 1-2 u 8 con plasma de otros hemofilicos se comporta como plasma normal como si las globulinas de Patek y Taylor de estos dos tipos de hemofilicos constituyeran fracciones complementarias diferen

PAVLOVSKY A, CASTELLANOS H y MITTELMAN D G de Comparación de la actividad aceleradora de la coagulación de la fracción globulina separada de los distintos hemofílicos Comunicación reunión anual de Inst Inv Fis Apl Pat Humana 1948
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VII 2

The Proconvertin-Convertin System and Its Significance for the Study of Hemophilia

P A OWREN*

HEMOPHILIA has probably existed in the human race before history began but it was not recognized as a specific disease until about a hundred years ago It is characterized by a lifelong tendency to prolonged hemorrhage which may be dramatic and often tragic

The diagnosis of hemophilia is usually simple from the clinical picture and the hereditary pattern The most valuable laboratory finding is the prolonged whole blood coagulation time called attention to by A L Wright in 1893

A prolonged clotting time is not pathognomonic however and other hemorrhagic diseases occur as inherited anomalies pseudohemophilia parahemophilia (proaccelerin deficiency) and the congenital and hereditary proconvertin deficiency

We are still lacking specific methods symptoms or signs for the diagnosis of hemophilia and a number of other important problems also remain unsolved

We don t know the nature of the specific clotting disturbance in hemophilia Alexander Schmidt and Manteuffel as early as 1893 found evidence for the lack of zymoplastic material or thromboplastin in present terminology in hemophilic blood and that is about as far as we are today

Quick Stanley Brown and Bancroft in 1935 confirmed that in hemophilia the thromboplastin is deficient and Brinkhous (1939) came to the same conclusion But still we have many controversies concerning the exact nature of the thromboplastin defect in hemophilia This question has already been reviewed by Dr Pavlovsky He has also called attention to the possibility of disturbances in the vascular function as a contributing factor in the hemorrhagic episodes

The study of hemophilia has been hindered by the fact that no adequate method has been available for the quantitative determination of the active thromboplastin which is formed in shed blood Neither do we have any reliable

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ence of the many cases studied such as (1) the relative mildness of the congenital fibrinopenia as compared to hemophilia even in those cases that have a short clotting time (2) because of the long periods of well being up to 2 years in some of our cases with no bleeding episodes and yet with no variation in their prolonged clotting time (3) the existence of bleeding episodes even in those hemophiliacs whose clotting time has been maintained at a reduced level by means of daily blood transfusions (4) because of the fact that hemorrhagic episodes can be stopped by therapy directed exclusively against the vessels etc

All this seems to prove the existence of some alteration of the vessels such as increased fragility or permeability due to unknown factors

Our Concept of the Pathogenesis of Coagulation Disturbance

Castex and Pavlovsky *observed* a lengthening in the clotting time of hemophilic recalcified plasma when this is deprived of platelets and when the test is performed a few minutes after the sample is obtained If both plasmas with and without platelets are allowed to stand for a certain period of time it is sometimes observed that there is a spontaneous shortening of the clotting time which leads one to think that it cannot be due simply *to platelet disintegration*

In 1944 Pavlovsky and Simonetti studied the clotting activity of the globulin fraction of Patek and Taylor extracted from hemophilic plasmas This did not seem to correlate *with the clotting* time and was of little value except in a few cases Castex Pavlovsky and Simonetti observed the paradoxical fact that certain hemophilic plasmas with very prolonged clotting times were capable of shortening the clotting time of other hemophilic blood

Treatment

We recommend a hygienic moral and physical life together with prophylaxis against trauma

For the general treatment of bleeding episodes we recommend blood transfusions of fresh frozen or lyophilized plasma or normal anti hemophilic globulin Locally we recommend *compression* ligature of the vessels and very careful suture aided with the application of thromboplastin human milk human placenta rabbit brain and thrombin

Orthopedic Treatment

Temporary immobilization of the hemarthrotic joint in the position it adopts until the pain ceases Two words on the preparation of the human placenta powder the fresh placenta is deprived of its membranes finely ground and vacuum desiccated it is then pulverized with a hand grinder and kept in vacuum sealed ampules

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VII 2

The Proconvertin-Convertin System and Its Significance for the Study of Hemophilia

P A OWREN*

HEMOPHILIA has probably existed in the human race before history began but it was not recognized as a specific disease until about a hundred years ago It is characterized by a lifelong tendency to prolonged hemorrhage which may be dramatic and often tragic

The diagnosis of hemophilia is usually simple from the clinical picture and the hereditary pattern The most valuable laboratory finding is the prolonged whole blood coagulation time called attention to by A E Wright in 1893

A prolonged clotting time is not pathognomonic, however and other hemorrhagic diseases occur as inherited anomalies pseudohemophilia parahemophilia (proaccelerin deficiency) and the congenital and hereditary proconvertin deficiency

We are still lacking specific methods symptoms or signs for the diagnosis of hemophilia and a number of other important problems also remain unsolved

We don t know the nature of the specific clotting disturbance in hemophilia *Alexander Schmidt and Manteuffel as early as 1893 found evidence for the lack* of zymoplastic material or thromboplastin in present terminology in hemophilic blood and that is about as far as we are today

Quick Stanley Brown and Bancroft in 1935 confirmed that in hemophilia the thromboplastin is deficient and Brinkhous (1939) came to the same conclusion But still we have many controversies concerning the exact nature of the thromboplastin defect in hemophilia This question has already been reviewed by Dr Pavlovsky He has also called attention to the possibility of disturbances in the vascular function as a contributing factor in the hemorrhagic episodes

The study of hemophilia has been hindered by the fact that no adequate method has been available for the quantitative determination of the active thromboplastin which is formed in shed blood Neither do we have any reliable

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method for the quantitative determination of the so called anti hemophilic plasma factor or factors. The prothrombin utilization test introduced by Brinkhous (1939) has certainly been of some value in disclosing the clotting disturbance in hemophilia. The test is not specific however because a delayed conversion of prothrombin to thrombin may occur by any disturbance in the complex of factors converting prothrombin to thrombin. Quick's modification of the prothrombin consumption test is even less specific because the so-called "prothrombin time" found in serum by Quick's method depends on several factors beside prothrombin, especially on convertin and accelerin.

The discovery of previously unknown clotting factors and new knowledge concerning the various reacting substances and their interactions has provided new technics for analyzing the reactions taking place during the clotting of blood. I shall here restrict my discussion mainly to the new factors *proconvertin* and *convertin* and shortly outline how this new knowledge may be of importance in approaching the problem of hemophilia. For an initial information I am presenting a diagram illustrating the clotting theory (fig. 1).

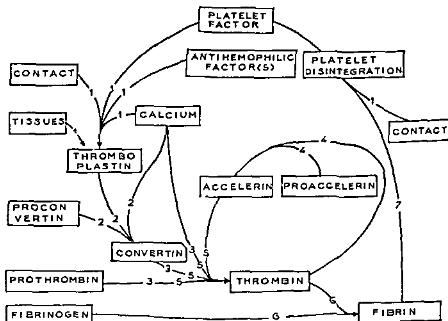


FIG. 1.—(1) Tissue injury yields thromboplastin directly while contact causes disintegration of platelets and release of a platelet factor which together with the antihemophilic factors (probably two different plasma factors) in the presence of calcium and contact give thromboplastin. (2) Thromboplastin and proconvertin in the presence of calcium forms convertin. (3) Convertin together with calcium brings about a minimal conversion of prothrombin to thrombin. (4) This initially formed thrombin starts the accelerator system, i.e. the conversion of proaccelerin to accelerin. (5) Accelerin accelerates the conversion of prothrombin to thrombin in the presence of convertin and calcium. (6) Thrombin converts fibrinogen to fibrin. (7) Fibrin provokes the disintegration of the platelets with further release of the platelet substance already mentioned. (The inhibitors antithrombin, antiaccelerin, anticonvertin, antithromboplastin and heparin are not included in the diagram.)

Because of the widespread use of a varying terminology on one and the same clotting factor I will make a few comments. *Proaccelerin* is the definite term first proposed by Astrup (1950) for the clotting factor which I discovered in 1943 and which was then provisionally named the fifth clotting factor or factor V. This factor was discovered by investigations in a case of congenital hemorrhagic disease which was shown to be caused by the lack of this previously unknown clotting factor. The deficiency disease was named parahemophilia. The new factor proaccelerin was concentrated from normal plasma and it was partly purified in a state free from admixture with other clotting factors. Many of its properties were examined and its action in the clotting process analyzed (Owren, P. A. 1944, 1947 a, b, 1948).

Proaccelerin itself was shown to be an inactive substance, a precursor or proenzyme which is activated during the clotting process. The active principle which has been termed *accelerin* governs the velocity of prothrombin activation. Ware and Seegers (1947) first demonstrated that the activation of proaccelerin to accelerin is caused by thrombin. Seegers and coworkers are using the terminology plasma Ac-globulin and serum Ac-globulin. From the data they have presented, however, it must be concluded that these factors are identical with proaccelerin and accelerin respectively.

In 1943, the same year that I observed my patient with parahemophilia, Quick demonstrated findings indicating that prothrombin is a complex composed of two factors, prothrombin A and prothrombin B. He later changed the term prothrombin A to the labile factor (1947). This factor of Quick is also identical with proaccelerin. The active form, accelerin, has not been discussed by Quick. The prothrombin conversion factor of Fantl and Nance (1946, 1948) and the cofactor of thromboplastin described by Honorato (1947) are also both identical with proaccelerin.

Proconvertin is another new clotting factor which I first reported in 1947 and which was then provisionally named Co-factor V. Experiments in 1945 to 1947 indicated the existence of this second accessory factor which is necessary for the conversion of prothrombin to thrombin. It was found that different prothrombin preparations of the same concentration varied greatly in the velocity of thrombin formation even when the conversion conditions with respect to thromboplastin, calcium and proaccelerin were quite identical. This varying convertibility was explained by various contamination of the prothrombin preparations by an unknown converting factor which was adsorbed together with prothrombin and followed prothrombin during preparation. It was not until 1949, however, that we succeeded in the fractionation into two components of a crude prothrombin prepared from ox plasma, one factor being the converting factor, proconvertin, the other factor being prothrombin itself (Owren and Bjerkelund 1949).

The existence of *convertin*, the active principle which occurs during clotting and which is found in serum, was also suggested by earlier experiments (Owren 1947). It was demonstrated that during clotting a new principle is formed which was provisionally named *factor VI* and which is the really active principle in

converting prothrombin to thrombin A high activity of factor VI was demonstrated in fresh normal serum which also contains accelerin Accelerin alone, however produced by adding thrombin to proaccelerin, did not show any factor VI activity This finding indicated that serum contains an additional factor to accelerin to constitute the complete prothrombin converting principle, previously named factor VI The existence of this additional factor convertin was confirmed by experiments on plasma from parahemophilia where there is no disturbance caused by the proaccelerin accelerin system During incubation of this proaccelerin free plasma with thromboplastin and calcium there appeared a progressive increase in activity of a principle which increased the rate of thrombin formation by the addition of accelerin (Owren 1950)

Various investigators have suggested the existence of clotting factors identical with the proconvertin convertin factors Hurn, Barker and Mann (1947) first called attention to the phenomenon that the two stage technic of prothrombin determination results in higher values than the one stage method by the testing of dicumarol plasma Mann and co workers (1949, 1951) also demonstrated that preliminary mixing of tissue thromboplastin with diluted plasma or serum potentiates its activity The principle which increased the activity of thromboplastin was found to be reduced in dicumarol plasma It was regarded as part of the thromboplastin complex and was designated "co thromboplastin activity"

We have found that dicumarol decreases the concentration of proconvertin simultaneously with prothrombin (Owren 1950) and it seems to be no doubt that the "co thromboplastin activity" of Mann is identical with proconvertin The "prothrombin conversion accelerator" of Owen and Bollmann (1948) and the "kappa factor" of Dam and co workers (1948) which also was found to be decreased in dicumarol plasma are probably both identical with proconvertin

Alexander and co workers (1948 1949 1950) have made extensive studies on a prothrombin converting factor which was found in serum and which was demonstrated to be different from proaccelerin and accelerin The factor was termed "the serum prothrombin conversion accelerator" (SPCA) The effect observed by Alexander is caused by proconvertin and convertin SPCA prepared from serum usually contains a mixture of proconvertin and convertin depending on the type of serum used

Koller (1951) introduced the name factor VII instead of proconvertin He applied our technic for the assay of this factor He did not find any evidence for the existence of the active principle convertin however A large number of other investigations have also relation to the proaccelerin accelerin system or the proconvertin convertin system but can not be discussed here

The problem of hemophilia resides in the initial reactions which result in the formation of active thromboplastin As pointed out by Pavlovsky hemophilia may be caused by a deficiency of one of the so called antihemophilic factors (it is probably two different plasma factors which together with a platelet factor take part in the formation of active thromboplastin) or by an increased activity of an inhibitor an antithromboplastin or antiCephalin as maintained by Tocantins I shall not discuss these problems at present but may mention our

finding pointed out in the diagram (fig 1) that contact has a decisive influence on the formation of active thromboplastin also *after* the platelet substance is released from the platelets

It follows from figure 1 that a prerequisite for analyzing the formation of active thromboplastin during clotting is the knowledge of the interaction of thromboplastin and proconvertin in the formation of convertin and methods which provide a possibility to follow these reactions quantitatively I therefore proceed to this problem

Methods have now been worked out for the specific and quantitative determination of proconvertin prothrombin and proaccelerin (Owren and Aas 1951) The principle underlying these methods is the use of a clotting mixture in which all factors are kept constant except the one to be determined The clotting time of such a mixture or reagent will then be dependent entirely upon the amount of the lacking clotting factor which is added Based on the final and main reaction in the thrombin formation (the conversion of prothrombin to thrombin by convertin and accelerin), methods have also been worked out for the specific determination of convertin and accelerin and a new method for prothrombin determination For the determination of convertin the clotting mixture used contains an excess of accelerin and prothrombin For the determination of accelerin a reagent is used which contains prothrombin and convertin and for the determination of prothrombin the reagent contains accelerin and convertin Thromboplastin is not used in any of these methods The preparation of the various reagents used in these methods and the technical details are published previously (Aas 1952 Owren 1952) and will not be discussed here

The application of these various methods for analyzing what happens during clotting of blood is illustrated by the following diagrams

Figure 2 illustrates the clotting of normal blood Convertin which is formed

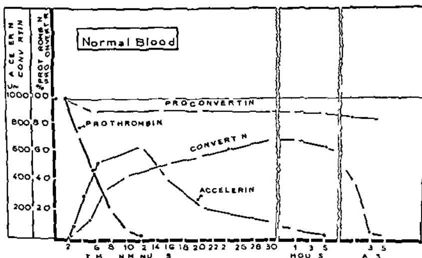


FIG 2 —The consumption of proconvertin and prothrombin and the formation and inactivation of convertin and accelerin during and after clotting of normal blood

converting prothrombin to *thrombin* A high activity of factor VI was demonstrated in fresh normal *serum which also* contains accelerin Accelerin alone however produced by adding *thrombin to proaccelerin*, did not show any factor VI activity This finding *indicated that* serum contains an additional factor to accelerin to constitute the complete prothrombin converting principle previously named factor VI The *existence of this* additional factor, convertin was confirmed by experiments on plasma from parahemophilia where there is no disturbance *caused by* the proaccelerin accelerin system During incubation of this proaccelerin free plasma with *thromboplastin and* calcium there appeared a progressive increase in activity *of a principle* which increased the rate of thrombin formation by the addition of accelerin (Owren 1950)

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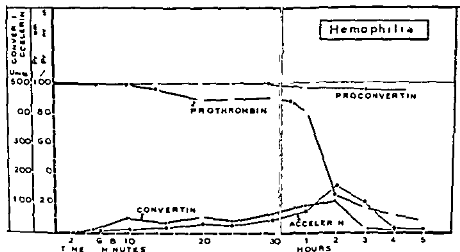


FIG 4 —In hemophilia both the convertin formation and accelerin formation takes place slowly A minimal amount only of proconvertin is utilized and the prothrombin consumption takes place slowly

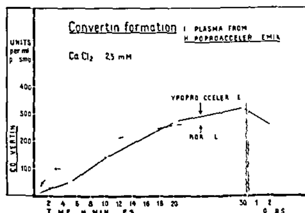


FIG 5—The convertin formation follows the same curve in proaccelerin deficient plasma as in normal plasma

on the presence or not of proaccelerin The formation of convertin takes place in the same way in a case of *proaccelerin-deficiency* as in normal blood (fig 5) The same holds true also by the addition of tissue thromboplastin to normal plasma and to plasma from congenital hypo proaccelerinemia (fig 6) When *proconvertin* is *lacking* however as shown by the lowest curve the formation of convertin also is deficient

Figure 7 illustrates that there exists a quantitative relationship between the amount of proconvertin present and the amount of convertin formed By high concentrations of thromboplastin and low concentrations of proconvertin we find an approximately linear relationship between the proconvertin concentration and the convertin formed

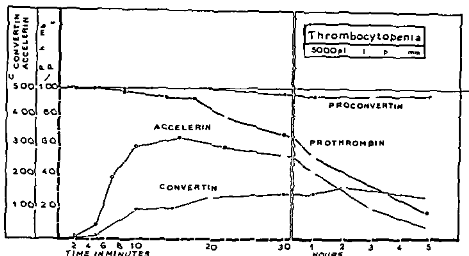


Fig 3—In thrombocytopenia the convertin formation takes place slowly and a small amount only of proconvertin is utilized

by the interaction of thromboplastin and proconvertin increases in activity during the first hour then decreases slowly and disappears completely in a week's time Owing to the low concentration of the active thromboplastin which is formed during clotting of normal blood a small amount only of the original proconvertin in the plasma *is consumed during the convertin formation* and 70–90% remains in serum This residual proconvertin in serum is very stable on storage Accelerin which is formed from proaccelerin by the action of thrombin increases rapidly as a consequence of the first thrombin formation and reaches a maximum in the first 15 minutes It is then inactivated and reaches a low level in 5–6 hours It disappears completely in 24–48 hours depending on how the blood and serum is handled Prothrombin decreases rapidly during and after clotting because it is converted to thrombin by convertin and accelerin *with subsequent inactivation of the thrombin formed*

Figure 3 illustrates the situation in *thrombocytopenia* Owing to the reduced formation of active thromboplastin caused by a deficiency of the platelet substance the formation of convertin takes place more slowly than in normal blood Consequently the consumption of proconvertin is reduced and the prothrombin utilization also takes place slowly The accelerin formation however is about normal because very small amount only of thrombin are needed for a complete activation of proaccelerin to accelerin

In hemophilia (fig 4) the formation of convertin takes place *very* slowly indicating a very reduced activity of the thromboplastin formed It is further seen that the thrombin formation in this case is so limited that it is insufficient for complete activation of *proaccelerin to accelerin The slow thrombin formation* is also reflected in the very slow disappearance of prothrombin

The addition of tissue thromboplastin eliminates the difference between normal and hemophilic plasma and the convertin curves are becoming equal

The next two figures illustrate that the formation of convertin is independent

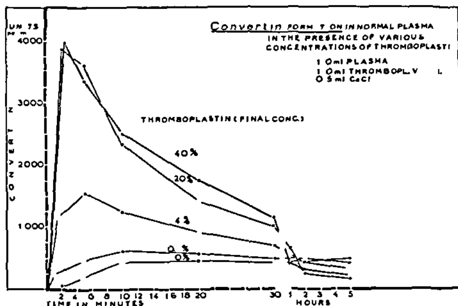


Fig 8 —By constant proconvertin concentration the activity of convertin increases with increasing amounts of thromboplastin up to a certain limit

For the study of hemophilia however it is of greater interest to analyze the *quantitative relationship between the thromboplastin concentration and the convertin formed*

By adding thromboplastin in various concentrations to normal plasma it is found that the velocity of convertin formation as well as the maximal amount formed increases with increasing concentrations of thromboplastin up to a certain limit (fig 8) Addition of thromboplastin above this limit has no influence This limit depends on the amount of proconvertin present

When proconvertin is present in relatively high concentrations and thromboplastin is added in low concentrations we have found an approximately linear relationship between the thromboplastin concentration and the maximum of convertin formed This situation corresponds to the relative concentrations in normal and pathological blood When the proconvertin concentration is normal therefore the convertin formation may be used as a measure of thromboplastic activity The method of convertin determination may consequently be applied as a new tool for research on hemophilia

The method of prothrombin utilization which has hitherto been applied is less useful because the conversion of prothrombin to thrombin depends on the combined effect of convertin and accelerin and in this reaction the effect of accelerin plays a most important role with respect to the velocity of the reaction A normal concentration of accelerin compensates a reduced formation of convertin This is illustrated in hypoproconvertinemia where the prothrombin

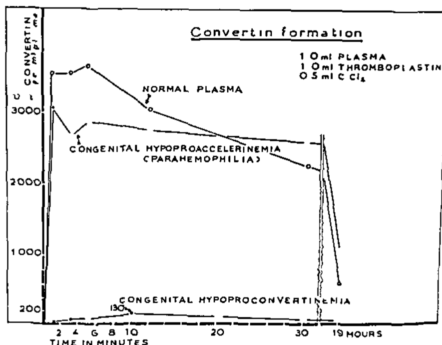


Fig 6 — The convertin formation in proaccelerin deficient plasma and in normal plasma is identical also by the addition of thromboplastin In proconvertin deficient plasma the convertin formation is very low

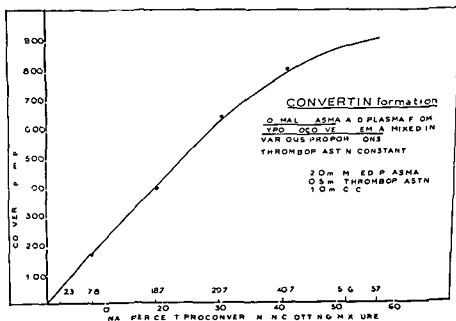


Fig 7 —The activity of convertin increases with increasing amounts of proconvertin

contiene protrombina y convertina Y para la determinación de la protrombina el reactivo contiene accelerina y convertina En ninguno de estos métodos se usa tromboplastina

La fig 2 muestra la coagulación de sangre normal La convertina que se forma de la inter acción de tromboplastina y proconvertina aumenta en actividad durante la primera hora y luego decrece lentamente desapareciendo completamente en una semana La accelerina que se forma de la pro accelerina por la acción de la trombina aumenta rápidamente como consecuencia de la primera formación de trombina llegando a su máximo en los primeros 15 Es luego inactivada llegando a bajos niveles en 5 a 6 horas y desapareciendo completamente en 24 a 48 horas La protrombina disminuye rápidamente durante y después de la coagulación porque es convertida en trombina por la convertina con subsiguiente inactivación de la trombina formada

La fig 3 muestra la situación en la trombocitopenia La formación de tromboplastina activa está reducida y la formación de la convertina es lenta La consumición de la proconvertina está reducida y la utilización de la protrombina es lenta La formación de accelerina es normal porque sólo se necesitan pequeñas cantidades de trombina para una completa activación de pro accelerina a accelerina

En la hemofilia fig 4 la formación de convertina se hace muy lentamente debido a una muy reducida actividad de la tromboplastina formada La adición de tromboplastina tisular elimina la diferencia entre plasma hemofílico y plasma normal y las curvas de convertina se igualan

Las dos figuras que siguen muestran que la formación de convertina es independiente de la presencia o ausencia de pro accelerina Cuando la proconvertina falta la formación de convertina también es deficiente

La figura 7 muestra que existe una relación cuantitativa entre la cantidad de proconvertina presente y la cantidad de convertina que se forma

Para el estudio de la hemofilia es de gran importancia analizar la relación cuantitativa entre la concentración de tromboplastina y la formación de convertina

La velocidad de formación de convertina como así también la máxima cantidad obtenida aumenta con la adición de mayores concentraciones de tromboplastina hasta un cierto límite (fig 8) La adición de tromboplastina por encima de este límite no tiene ninguna influencia

Cuando la concentración de proconvertina es normal la formación de convertina puede usarse como una medida de la actividad tromboplástica La determinación de la formación de convertina puede por lo tanto usarse como un nuevo test para el estudio de la hemofilia

Hasta la fecha hemos estado investigando la inter acción de la proconvertina tromboplastina y la formación de convertina Las investigaciones siguen progresando aplicando nuestros nuevos métodos en el estudio del trastorno de la coagulación en la hemofilia

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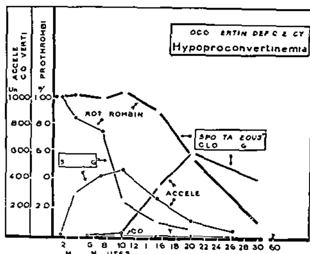


FIG 9—In hypoproconvertinemia the initial prothrombin consumption is slightly delayed but follows otherwise a normal curve

utilization is about normal in spite of the fact that the convertin concentration is very low (fig 9)

Up to the present time we have been engaged in investigations on the proconvertin thromboplastin interaction and the formation of convertin Research is in progress applying our new methods in the study of the hemophilic clotting disturbance

EL SISTEMA PROCONVERTINA CONVERTINA Y SU SIGNIFICACION EN EL ESTUDIO DE LA HEMOFILIA

El problema de la hemofilia reside en las reacciones iniciales que resultarán luego en la formacion de tromboplastina activa

Como lo ha señalado Pavlovsky la hemofilia puede ser debida a la deficiencia de uno de los llamados factores anti hemofílicos (sería probablemente debida a dos factores plasmáticos distintos los cuales junto con un factor plaquetario tomarían parte en la formacion de tromboplastina activa) o a una actividad aumentada de un inhibidor que podría ser una anti tromboplastina o una anti cefalina segun mantiene Tocantins

No entraré a discutir estos problemas pero quiero recalcar que en nuestra experiencia y segun muestra el diagrama que sigue (fig 1) el contacto tiene una influencia decisiva en la formación de tromboplastina activa aun después de que la substancia plaquetaria ha salido de la plaqueta

Se desprende de la fig 1 que un requisito necesario para analizar la formación de tromboplastina activa durante la coagulacion es el conocimiento de la inter accion de tromboplastina y proconvertina en la formacion de convertina como así también los métodos mediante los cuales estas reacciones pueden medirse cuantitativamente

Estos métodos han sido hoy día perfeccionados pudiendose obtener determinaciones específicas y cuantitativas de proconvertina protrombina y proacelerina (Owren y ... 1951) El principio básico de estos métodos es el uso de una mezcla coagulante en la cual todos los factores se mantienen constantes excepto aquel que se quiere determinar El tiempo de coagulacion de estas mezclas dependerá exclusivamente sobre la cantidad que debe agregarse del factor que falta

Para la determinacion de la convertina la mezcla coagulante contiene un exceso de accelerina y protrombina Para la determinacion de la accelerina se usa un reactivo que

la categoría de ley Lossen[3] en 1876 fué más allá todavía y sostuvo que el hombre hemofílico nunca transmitía la enfermedad

Estos conceptos ya sea totalmente o en parte son todavía aceptados en la actualidad a pesar de que estudios tan tempranos como los de Hay (1810) claramente mostraron transmisión de la enfermedad por vía masculina y de la existencia de casos citados en la literatura, de mujeres con tendencia hemorragica en familias hemofílicas[5]

Bauer[6] tratando de explicar la escacez o ausencia de la hemofilia en mujeres sugirio que esta situacion solo ocurriría en condiciones de homocigocitosis (dos cromosomas X defectuosos) y de que dicha condicion sería de consecuencias letales para la descendencia

A pesar de que los tempranos investigadores no contaban con métodos adecuados para la investigacion de portadores o de situaciones de héterocigocitosis el concepto de la hemofilia como enfermedad unicamente transmitida por mujeres ha sido en varias ocasiones puesto en duda como bien lo sostiene Gates[5] Mas recientemente Merskey[7] ha hecho una puesta al dia en los estudios familiares que primeramente iniciara Treves en 1886 en una familia de hemofílicos Cuatro mujeres en la 5 generacion cuyos padres eran primos hermanos tienen clinicamente el cuadro de dicha enfermedad confirmado por pruebas de laboratorio Israels[8] también ha descripto casos de hemofilia en mujeres

La falta de una prueba de laboratorio específica para la hemofilia y en otros casos los menos la falta de acuerdo sobre un criterio clinico específico ha sido principalmente la causa de dudas y confusiones El abandono de los principios clásicos en esta enfermedad especialmente al entrar en el terreno de hemofilias femeninas y portadores masculinos ha sido por lo general observado con escepticismo

El defecto o los defectos causantes de la enfermedad no han sido claramente establecidos

El rol del factor anti hemofílico del plasma (globulina anti hemofílica) la anti tromboplastina y los posibles factores vasculares necesitan todavía mayor estudio Por el otro lado Brinkhous[9] ha demostrado en forma bastante convincente que en la hemofilia canina la enfermedad esta verdaderamente relacionada con un caracter sexual recesivo y que sigue la distribucion clasica o sea de que aparece en los machos y en el caso de las hembras solo aquellas homocigoticas al gen defectuoso la sufren

La posibilidad de la aparicion espontanea de la hemofilia debido a mutacion genetica ha sido considerada por varios autores Haldane[10] sugirio que la mayoria de los genes hemofílicos sub letales en los cromosomas X presentes en individuos de sexo masculino desaparecerian en cada generacion y de que seria necesaria una secuencia mutacional de 1 en 50 000 para mantener la incidencia de la hemofilia en la poblacion de Londres El mismo autor tambien sostiene que la probabilidad para la aparicion del fenomeno de mutacion en una familia dada es mayor cuanto mayor es el numero de individuos normales del sexo masculino en las generaciones anteriores Boggs[11] por ejemplo ha recogido varios casos de hemofilias espontaneas en la literatura y describe el caso de una

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VII 3

Estudios sobre Herencia en Hemofilia Mediante Metodos de Laboratorio

JOSEPH M HILL, G CRASS JOHN ELLIS y K P WITTSTRUCK*

EL USO satisfactorio de las pruebas de laboratorio para la determinacion de héterocigocitosis y homocigocitosis en la herencia de los factores Rh sugirio la posibilidad de que tales métodos de investigacion pudieran también ser aplicados para determinar relaciones análogas en la hemofilia Dicha informacion de obtenerse podria ser de considerable valor clínico en la busqueda de portadores femeninos y arrojaría luz sobre el problema de hemofilias en mujeres portadores masculinos y hemofilias parciales o héterocigoticas en ambos sexos

Ha sido comunmente aceptado hasta la fecha que la hemofilia se hereda como un caracter sexual recesivo

Otto[1] quien describio la enfermedad en 1803 noto que la tendencia hemorragica solo se hacía aparente en el sexo masculino y que las mujeres si bien no la sufrían eran capaces de transmitirla a sus hijos varones Nasse[2] dio a esta teoría

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I 30%

II

III 15% 100%

IV 100%

% Prothrombina no consumida

Sexo masculino

Sexo femenino

Hemofilia parcial

Hemofilia clínica

Con estudios de laboratorio

Sin estudios de laboratorio

Fig 1

Fig 2

tiempos de coagulación anormales (135 y 180 minutos) y tiempos de coagulación del plasma recalcificado típicos después de centrifugación lenta y rápida (lenta 193 segundos y rápida 1021 segundos para un caso y 162 segundos y 790 según dos para el otro)

En esta familia vemos también un cuadro de deficiencias parciales en la utilización de la protrombina tanto en varones como en mujeres con una incidencia sorprendentemente alta de tales defectos en los parientes del padre y de la madre de los individuos hemofílicos

16 de los 31 individuos estudiados mostraron valores por encima del 15% mientras 12 de ellos mostraron cifras del 20% o más de protrombina no consumida La presencia de esta alteración en la hermana madre y abuela de los 2 hemofílicos típicos sugirió que las portadoras estaban siendo posiblemente

familia en la cual se encontraron 6 hemofílicos varones sin existir ninguna historia de tendencia hemorrágica en la ascendencia directa por vía materna, como así tampoco en la paterna a pesar de que el estudio de dicha familia incluyó 5 generaciones de familias numerosas

Metodos

Para evitar en lo posible caer en situaciones que se prestarían a discusiones, decidimos empezar en nuestros estudios, tomando casos de hemofilia típicos tanto clínicamente como mediante pruebas de laboratorio Todos los familiares accesibles fueron entonces estudiados usando las siguientes pruebas de laboratorio recuento de plaquetas, tiempo de sangría tiempo de coagulación (metodo de Lee y White), tiempo de protrombina (en una etapa) consumicion de protrombina (una etapa) y tiempo de coagulación del plasma recalcificado después de centrifugación rápida y lenta

En adición a esto, se hicieron estudio de antígenos eritrocitarios incluyendo los grupos A_1 B C D E c C^w d, e, M, N P Lu, Le y Kell para estudiar las correlaciones familiares y despistar casos de falsa paternidad

La prueba de la consumicion de protrombina (una etapa) se hizo siguiendo exactamente la técnica descripta por Quick [12] La sangre se obtuvo por puncion venosa con agujas y jeringas siliconizadas y depositada en tubos de ensayo de 13 x 100 mm limpios y tambien siliconizados para su coagulacion

Inicialmente el suero usado para las pruebas se obtuvo después de 1 3 y 24 horas de colectada la sangre pero mas tarde utilizamos como procedimiento "standard" el suero de 24 horas debido a los resultados mas uniformes obtenidos mediante esta tecnica comparada con los sueros controles Para obtener las cifras de variacion normal la prueba se hizo sobre 100 muestras de sangre consecutivas obtenidas de dadores en el banco de sangre, con una prueba adicional sobre 10 mujeres embarazadas del servicio de maternidad

Resultados

La serie de pruebas de consumicion de la protrombina (una etapa) sobre 24 horas efectuada en las muestras normales colectadas para la prueba mostro una cifra del 5% al 10% de protrombina residual (67 a 40 segundos) en los dadores y del 2% al 5% en las mujeres embarazadas Se extendio arbitrariamente como cifra límite normal el 15% Del 15% al 20% se considero probablemente anormal y por encima del 20% evidentemente anormal

En la figura 1 la herencia parece seguir la distribucion clasica y si aceptamos la cifra de 15% para la protrombina no consumida en 24 horas como anormal vemos que las mujeres portadoras podrían haber sido despistadas con esta técnica

La figura 2 muestra un estudio familiar donde 31 individuos en 3 generaciones fueron estudiados con pruebas de laboratorio completas

De los 3 varones hemofílicos uno habia fallecido pero los dos estudiados eran hemofílicos típicos con un déficit completo en la absorcion de la protrombina en el proceso de coagulacion en 24 horas Estos dos casos mostraron también

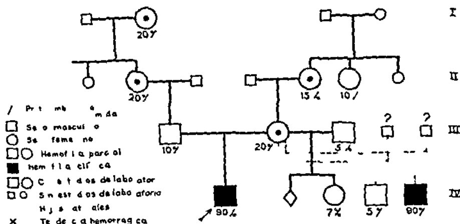


Fig 5

Las figuras 4 y 5 representan estudios en una misma familia de raza negra. En la figura 4 el "pedigree" aparece según relación de la madre pero la figura 5 muestra el verdadero árbol genealógico según lo demostraron los estudios con antígenos eritrocitarios.

La madre prontamente contestó al interrogatorio concerniente a su familia y aseguró la ausencia de casos de hemofilia conocidos previos a los de sus hijos. Durante su presente matrimonio tenía 2 hijos, ambos hemofílicos. Previo a esto había tenido otros 2 hijos varones con otro marido, uno de ellos vivo y sano, el otro fallecido pero no de hemofilia. En adición a esto tenía un tercer hijo varón sano con un tercer hombre. Los estudios serológicos mostraron sin embargo que uno de los hijos hemofílicos no podía provenir de *ninguno* de los 2 maridos, un hecho interesante si se considera la rareza de los genes hemofílicos especialmente en la raza negra.

Este caso pone en evidencia los inconvenientes que pueden presentarse al hacer estudios genéticos de cualquier enfermedad, aun cuando los individuos interrogados tratan de dar la verdadera historia. En este caso nuevamente encontramos por ambos lados de la familia un alto porcentaje de individuos con defectos en la utilización de la protrombina (6 de 10 incluyendo a los 2 hemofílicos) pero ninguna correlación significativa con los tiempos de coagulación. La madre dió una historia de epistaxis antes de la pubertad y la abuela una historia de menorragia y hemorragia abundante post extracción dentaria.

La figura 6 muestra otro estudio en una familia donde previamente no hubo ningún caso de hemofilia conocido. Un hijo había fallecido de hemofilia y el otro era clínicamente un caso típico de hemofilia severa.

13 de los 18 individuos estudiados en esta familia mostraron una consumición anormal de protrombina y en este grupo se encontró un 100% de correlación de este defecto con un tiempo de coagulación prolongado (1 a 4 minutos por encima de la cifra normal de 15 minutos). En esta familia ambos padres tenían

Fig 3

*Pedigree Hemofilico Segun Historia Familiar

Fig 4

identificadas por esta prueba El padre de los hemofilicos sin embargo mostro pruebas de laboratorio normales

La figura 3 muestra el estudio de 35 individuos en 5 generaciones diferentes

El unico caso de hemofilia presento el cuadro clinico clasico y mostro un tiempo de coagulacion de 33 minutos con un 100% de protrombina no consumida en 24 horas El plasma recalcificado coagulo a los 190 segundos de pués de centrifugacion lenta y a los 360 segundos de pués de centrifugacion rapida De nuevo un gran porcentage de los parientes estudiados mo traron consumicion anormal de la protrombina (14 de 35)

Tanto el padre como la madre mostraron pruebas anormales De los 14 individuos con defectos en la consumicion de la protrombina 12 tambien presentaron tiempos de coagulacion prolongados y uno mo tro un tiempo de coagulacion prolongado con una utilizacion de protrombina normal

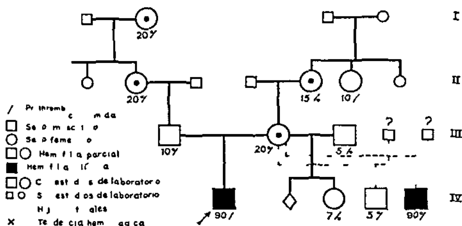


FIG. 5

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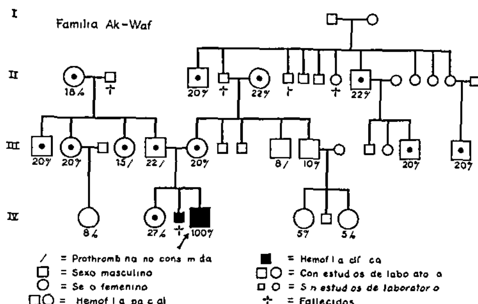


Fig 6

defectos medibles y una hermana del paciente mostro un 27% de protrombina no utilizada y un tiempo de coagulacion de 19 minutos 40 segundos pero sin ninguna historia de hemorragias

Otras 2 familias fueron estudiadas pero solo unos pocos de los parientes fueron localizados

El primer caso fué un varon de 11 anos de edad de raza negra cuya madre y abuela mostraron un 15% y un 30% de protrombina no consumida Un tío y dos tíos abuelos habían sufrido severas hemorragias

La segunda familia tenia 2 hijos varones con severa hemofilia sin ningun caso conocido de hemofilia en 4 generaciones Sin embargo una bisabuela (paterna) sufrio hemorragias abundantes pero dio a luz 8 hijos

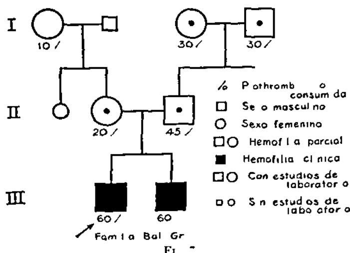


Fig 7

La abuela materna tenía equimosis con facilidad pero no dio ninguna historia de hemorragias

Los estudios de laboratorio mostraron un 15% de protrombina no consumida en el padre 20% en la madre y 30% en los dos abuelos paternos

Como resumen de estos hallazgos 7 familias fueron estudiadas en las cuales un total de 109 individuos tuvieron estudios de laboratorio completos El total de hemofílicos estudiados comprendió 10 casos 30 de cuyos parientes mostraron defectos parciales en la consumición de protrombina (15% o más de protrombina no consumida en el suero después de 24 horas de colectada la sangre) Los resultados de las demás pruebas de laboratorio no mostraron ninguna anomalía en cuanto al recuento de plaquetas tiempo de sangría o tiempo de protrombina

El tiempo de coagulación prolongado correlacionó bien con la hemofilia pero no dio resultados enteramente paralelos con la consumición de protrombina parcialmente anormal en los parientes

Lo mismo se observó con el tiempo de coagulación del plasma recalcificado después de coagulación lenta y rápida

Discusión

Estos estudios fueron destinados a recoger informaciones no tanto de los enfermos de por sí sino de las familias de los pacientes hemofílicos No fué nuestro propósito tratar de hallar una *nueva prueba de laboratorio específica para la hemofilia* sino de utilizar los métodos de análisis disponibles para descubrir peculiaridades en las familias de hemofílicos típicos tanto clínicamente como por investigaciones de laboratorio Al mismo tiempo hacemos notar que la prueba que resultó más útil para nuestro propósito o sea la prueba de la consumición de la protrombina en una etapa fue cuidadosamente standardizada sobre 100 dadores de sangre y 10 mujeres embarazadas Estos resultados mostraron una curva con un máximo de un 10% de protrombina no consumida después de 24 horas como índice de normalidad Tomando una actitud razonablemente conservadora fijamos el 15% de protrombina no consumida como límite mínimo de significación patológica

No queremos pretender con esto que la prueba de la consumición de la protrombina tiene un valor específico para las hemofilias o hemofilias parciales (si puede aceptarse tal denominación) En realidad esta prueba en una etapa apenas si puede considerarse específica para la protrombina desde el momento que ignora los aceleradores de la protrombina junto con otros factores importantes

Sin embargo bajo las condiciones en que se hicieron estos estudios y con las pruebas de laboratorio adicionales tales como el recuento de plaquetas determinación de la protrombina tiempo de coagulación y en algunos casos investigación de la globulina Ac creemos que los resultados se hicieron bastante específicos para nuestro propósito

Otro aspecto interesante de este trabajo fué el haber efectuado en forma extensiva el estudio de un gran número de antígenos eritrocitarios para controlar la exactitud de nuestros estudios genéticos Esto es de suma importancia en la investigación de las llamadas hemofilias espontáneas como lo indicara Boggs[11]

La utilidad de estas consideraciones *genéticas esta bien ilustrada en las figuras* 4 y 5 y al mismo tiempo asegura la exactitud de los demas estudios

Un numero sorprendente de individuos mostraron defectos parciales en las familias examinadas Sorprendente también fué la uniformidad de distribucion de tales defectos por las *dos ramas materna y paterna, de los enfermos estu*diados Tales hallazgos estan en cierto modo en oposición con los conceptos clasicos de la herencia en la hemofilia, pero concuerdan con las observaciones clínicas de distintos autores en el pasado y en la actualidad La hemofilia espontanea por ejemplo tiene *una mejor explicacion siguiendo estos conceptos* que aceptando una mutacion genética de 1 en 50 000 [10] Mas todavia, la herencia de defectos o defectos parciales por vía masculina, se hace ahora la regla en vez de la excepcion

La hemofilia en la mujer puede ahora ser considerada bajo otros puntos de vista La forma clinica severa es probablemente todavía el resultado de la coincidencia en la herencia de un grave defecto por vía materna y paterna conjuntamente Pero grados menores de tendencia hemorragica en mujeres parecen deberse a la herencia de un defecto que en los varones al menos es parte del cuadro de la hemofilia

¿Por que no podrian considerarse tales casos como hemofilias parciales o benignas?

Por el otro lado en estas familias también se encontraron defectos parciales en varones

Otra peculiaridad interesante de estos hallazgos es la de que el defecto parcial en la consumicion de la protrombina no siempre coincidio con una tendencia hemorragica ambos sexos considerados

Seria logico considerar entonces que tales defectos parciales en familias donde no existen casos conocidos de hemofilia serían la razon de algunos de nuestros problemas diagnosticos en enfermadades hemorrágicas tanto en varones como en mujeres

Finalmente creemos que deben hacerse estudios mas extensos en familias hemofilicas antes de modificar definitivamente las viejas teorías establecidas en cuanto a la herencia de esta enfermedad

Los estudios aquí presentados sugieren la posibilidad de la existencia de multiples factores en la herencia Como probablemente así también en el mecanismo de la hemofilia

Las nuevas informaciones que podremos obtener de los estudios de los coagulacionistas harian posible la aislacion de estos factores y el estudio de su herencia mediante adecuados *procedimientos de laboratorio* Tales *trabajos* como lo de Tocantins Owren y Seegers abren tales posibilidades

Nuestro trabajo sugiere la conveniencia de estudiar a los parientes tan cuidadosamente como a los pacientes hemofilicos para completar nuestros conocimientos en esta enfermedad

Una implicacion terapeutica como resultado de estos estudios sería la de evitar elegir a los parientes del enfermo así no presenten sintomatología alguna al elegir dadores de sangre en el caso de pacientes con tendencia hemorragica

STUDIES ON THE INHERITANCE OF HEMOPHILIA BY LABORATORY TESTS

Our studies on hemophilia have been carried out during the past several years and have dealt chiefly with the use of a laboratory test, namely, the Prothrombin Utilization Test, to determine possible abnormalities of the method in the families of hemophiliacs. While the test mentioned is not entirely specific for this disease, we believe that under the conditions of these studies, starting with known hemophiliac families, the test does become specific. Our work was an outgrowth of some studies on erythroblastosis and the determination of heterozygosity in the inheritance of the Rh antigens. The successful outcome of such experiments led our staff to consider the possibility that laboratory methods might reveal something about heterozygosity in the carrier female of hemophilia. Many of the blood clotting tests available at the time were applied to the examination of blood samples from families in which hemophilia occurred. The tests included bleeding time, clotting time, prothrombin levels, prothrombin utilization, platelet counts, Factor V (Ac globulin) and the slow and fast recalcification techniques. In addition to these tests the erythrocytes of each member of the family was studied serologically to determine the blood antigens. These studies afforded a means of lineage control and in two instances nonpaternity was uncovered. The sixteen antisera used for this study were Anti A, B, absorbed B, M, N, C, D, E, c, e, C, Lu, Le, and Kell.

In considering the well known and accepted genetics of hemophilia, one is soon faced with some of the statements that have been made by Haldane (50). He has calculated that on the average, a hemophilia gene would die out in approximately three generations in a stable population. However, Gates (51) reviews a number of instances in which hemophilia persists for more than three generations. If one third of the hemophilic genes are lost in each generation, one can conceive of the eventual disappearance of hemophilia. Haldane considered this possibility in the light of data that showed the incidence of hemophilia in London to be constant. He therefore surmised that the rate of mutation to hemophilia must be equivalent to the frequency of hemophilia in order to replace the lost hemophilic genes. He estimated the rate as approximately 1 in 50,000 in the London population. The evidence for such mutations is found in families with no obtainable history of hemophilia producing progeny having the disease. This would mean that there is a tendency for mutation of a normal gene resulting in the disease hemophilia. There are a number of instances in genetics in which a point mutation might explain such a situation. With these theories in mind, we began our studies on hemophilia with the hope of detecting the heterozygous female carrier. Some of the results can be seen in the charts. On chart 1 is seen a part of the family McW Jo in which hemophilia appeared without any previous history. The first progeny in the four generations shown resulted in two cases of hemophilia. The third generation had one case and the fourth generation also had one case.

When the various tests were applied to the available members of the family it was found that the mother of the first generation had a prothrombin utilization value of 30 per cent, 20 per cent greater than our normal range. In generation three, the female had a prothrombin utilization value of 35 per cent and the hemophiliac male showed 100 per cent unutilized prothrombin as did the male of the fourth generation. It was felt that the two females tested showed a defect that was detected by the Quick prothrombin utilization test. It is quite possible that this may be a test for heterozygosity and in this family it seemed to fit very nicely with the accepted theory of recessive sex linkage of hemophilia.

There was a carrier female, an untested male and a progeny containing two hemophiliacs. We were unable to obtain blood from the second generation female and the male she married but as far as we knew he had no history of hemophilia in his family. This family is typical of hemophilia and is quite representative of what we found at first. However, when other families were studied more completely, the prothrombin utilization data became significant.

On chart 2 is family Max Fox. The data shown covers four generations with thirty six individuals having been studied. Of these, two were clinical hemophiliacs and fourteen

showed abnormally high unutilized prothrombin by the Quick prothrombin utilization test One male had died of hemophilia before this study began In this family one can readily observe the prothrombin utilization abnormality on both sides of the family Both the grandmother and mother of the hemophiliacs had the defect On this chart it will be noted that whereas the hemophiliacs ran 100 per cent unutilized prothrombin the affected but non hemophilic relatives ranged from approximately 15 per cent to 30 per cent unutilized prothrombin In our study on normals we ran a series of blood bank donors and found that the normals ran from between 5 and 10 per cent by our tests We arbitrarily added another 5 per cent and used 0 to 15 as our normal range

The data presented on chart 3 shows a Negro family in which hemophilia occurred This chart shows the family tree based on information obtained from members of the family The prothrombin utilization results and the detailed blood group data are given

There was no previous history of hemophilia in this family The sudden appearance of a hemophiliac fits with the literature on the rareness of this disease in the Negro One might assume that in this family a mutation brought on this situation However when the prothrombin utilization data is examined one may readily see the defect on both sides of the family It is also interesting to note that the supposed father of the hemophiliacs had a normal prothrombin utilization while the defect was present in his mother and grandmother The marriage with a female having the defect resulted in hemophilia

The normal prothrombin utilization of this father along with other such instances has made us consider the possibility of another substance having normalizing or neutralizing effect in the case of carriers The presence of this gene (neutralizing) might suppress the appearance of the prothrombin utilization defect

The history of this family is somewhat amusing The mother was free with her information and readily answered any questions concerning her family and children Her present marriage resulted in two children both of whom were hemophiliacs Before this marriage she had another husband That marriage resulted in two children one normal and living and the other died of causes unknown but apparently not hemophilic In addition the mother admitted extramarital relations with a third man by whom she had a normal son

The propositus (the child brought in for treatment which led to the family study) could be the offspring of the second marriage but the other hemophilic child could not be the issue of the present husband or the previous one Consequently we must assume that he was either the son of the admitted paramour or possibly of a fourth man. This case emphasizes one of the dangers that is faced when one is doing genetic studies on any disease

The prothrombin utilization test in our hands seems to be an indicator of the carrier state of a clotting defect associated with the disease hemophilia The finding of this defect in both males and females raises certain questions on the inheritance of hemophilia in man If these findings are valid then it would be difficult to call hemophilia a sex linked disease There are two other types of inheritance that should be considered one a sex influenced character and the other a sex limited character

These studies have considerable clinical significance in that they indicate first that while full blown clinical hemophilia seems to occur only in males a partial defect can occur both in females as well as males This partial defect may or may not be associated with a tendency toward hemorrhage These findings also throw new light on the heredity of hemophilia and from the clinical side at least indicate that there may be partial defects in the families of hemophiliacs without frank clinical hemophilia or even detectable bleeding tendencies being known This is of some importance in the taking of history in the diagnosis of this disease

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VII 4

Activation of Purified Prothrombin with Hemophilia Plasma

SHIRLEY A JOHNSON JULIUS RUTSKY CHARLES L SCHNEIDER and WALTER H SEEGERS*

OF THE early investigators to suggest that hemophilia was essentially not a disease of the blood platelets but instead a condition concerned with a deficiency of one of the plasma components of the blood Van Creveld[1] was perhaps first followed by Patek and Stetson[2] They showed that a certain fraction of normal plasma or serum would reduce the coagulation time of hemophilic blood Thus the concept that hemophilia might be due to a deficiency was put forth Many experiments since have led to the wide acceptance of this concept and others have introduced the possibility that such is not the case For example by meticulous laboratory methods Tocantins et al[3] have carried out experiments which make it necessary to consider that the abnormality in hemophilia may be due to an excess of an inhibitor of blood coagulation The inhibitor

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has been called antithromboplastin and found to be present in greater quantity in the plasma and other tissues of hemophiliacs. A most interesting experiment by Tocantins showed that a globulin from hemophilic plasma could cause clot acceleration of hemophilic blood just as well as a preparation from normal plasma. If hemophilic plasma is deficient in such a globulin it should be impossible to prepare active material from hemophilic plasma unless another similar substance is also present. The only escape from an abandonment of that basic idea is on a quantitative basis or hypothecating that something of additional importance is being overlooked.

Whatever the nature of the important plasma component may be, it may be regarded as having an important relationship to platelets. Views on this relationship include the concept of a thrombocytolysin.[4] It has also been postulated that platelets act in some way with a substance in plasma to assist in the activation of prothrombin.[5] Further elucidation of this showed that the combination of the platelets and the plasma factor furnishes the equivalent of thromboplastin. Graham, Buckwalter, Hartley and Brinkhous[6] and Graham, Penick and Brinkhous[7] have called the deficient plasma component in hemophilia the antihemophilic factor and have reported a method of assay. In Quick's[8] theory the hemophiliac *is regarded as deficient in thromboplastinogen. This is thought to be* a precursor substance in plasma which can be activated by platelets to produce an activator of prothrombin.

In this laboratory it has been shown that platelets contain an accelerator of prothrombin activation that acts much like serum Ac-globulin.[9, 10] It was referred to by the term platelet factor I or platelet AcG. Fractions of platelets that are rich in platelet AcG may act in conjunction with a platelet cofactor *from plasma* and calcium ions to activate purified prothrombin rapidly.[11] Neither the platelet AcG alone nor the platelet co-factor alone with calcium ions will activate purified prothrombin rapidly, but platelet AcG and platelet co-factor *together* activate purified prothrombin rapidly. Moreover, after the combination has activated purified prothrombin rapidly it can again be separated to give two fractions which alone do not activate purified prothrombin but can be combined a second time to function in the rapid activation of purified prothrombin.

In the work described below purified prothrombin was activated with preparations of platelet AcG in combination with hemophilic plasma. Platelet co-factor activity is found in hemophilic plasma but to a lesser extent than in normal plasma, its activity being almost the same as in normal human serum. When hemophilic patients were transfused with normal lyophilized human plasma the quantity of platelet co-factor activity, as measured in test tubes by our new technique, increased.

With the use of platelet AcG and platelet co-factor to supply the equivalent accelerator globulin and thromboplastin activity in the presence of calcium ions we have been able to activate rapidly purified prothrombin. The thrombin production was measured by determining the clotting time of partially purified fibrinogen.

Experimental Procedures

Purified Prothrombin Purified prothrombin was prepared by the method of Seegers and associates [11,12,13]

Platelet AcG As in previous work a platelet extract was made from bovine platelets They were obtained by differential centrifugation and washed three times with physiological saline solutions One part of packed platelets was then mixed with 3 parts of saline and frozen in a deep freeze Then 10 ml of suspension were thawed and centrifuged at approximately 1,500 g for 30 minutes in an angle head refrigerated centrifuge to remove suspended material The sediment was recovered washed once in physiological saline and resuspended in 2 ml of saline The preparation consists of discrete particles which have been found to contain in addition to cytoplasmic fragments some whole platelets when examined with a phase contrast microscope This resuspended sediment contains the activity described by Ware Fahey and Seegers as platelet AcG activity

Platelet Co factor Prepared from bovine and human plasma as previously described [11] In principle the globulin can be precipitated from diluted plasma In aqueous solutions it is soluble at pH 6 4 and can be precipitated at 56 per cent of saturation with ammonium sulphate

Preparation of Plasma Samples Both normal and hemophilic blood samples were collected and prepared in exactly the same way A clean vein puncture was made with a siliconed syringe and the first syringe was replaced after 2 ml were withdrawn and discarded by another Then 10 ml of blood were placed immediately in a siliconed centrifuge tube containing 1 ml of 0 112M potassium oxalate The two were mixed and centrifuged at 1,500 g at 5 C for 30 minutes The plasma was defibrinated by adding an equal volume of purified thrombin containing 20 units per ml To destroy antithrombin the preparation was then treated with an equal volume of ethyl ether and shaken for five minutes This was repeated three times The material was stored in small aliquots in a deep freeze

Acacia Calcium and Imidazole Buffer These were made as described by McClaughry and Seegers and others

Fibrinogen A 1 0 per cent solution of fibrinogen was prepared by the method described by Ware Guest and Seegers

Thrombin Activity Method of Seegers and Smith

Results

Assay of Platelet Co factor As shown in previous work when purified prothrombin is activated by a combination of calcium ions platelet AcG and a crude platelet co factor preparation thrombin is formed rapidly [11] There is always a latent period of thrombin production of 3 or 4 minutes duration This is followed by a more rapid activation of prothrombin and a full yield is not obtained until about 30 minutes had passed Thrombin is produced in smaller amounts when limited quantities of platelet co factor are used for activation Evidently limited quantities of activator produce limited quantities of thrombin and the amount and rate of formation of the latter can serve as a quantitative measure of the amount of co factor present

If in the place of partially purified platelet co factor defibrinated ether treated plasma was used a similar activation of purified prothrombin was obtained Again the concentration of thrombin and its rate of production were limited if the amount of plasma in the mixture was limited This means that the basic fundamentals can be applied to plasma samples for assay purposes

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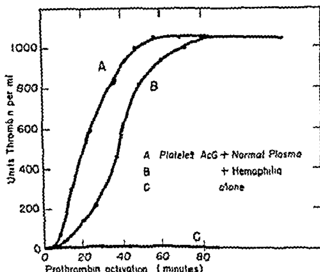


FIG 2 — Activation of purified prothrombin with platelet AcG and plasma diluted 1 1? The 3 year old hemophilia was given 100 cc of reconstituted dried plasma and the plasma sample for analysis was taken within thirty minutes after the infusion

conversion was more rapid (fig 2 curve C) but still slower than with normal plasma (fig 2 curve A) The work was confirmed on another case

Assay of Platelet Co factor in Serum Brinkhous[7] and associates have observed that the antihemophilic factor is not found in serum by their assay technique This finding made it of interest to examine serum in our procedure Human

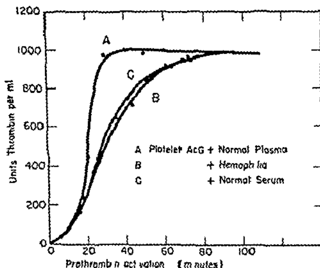


FIG 3 — Activation of purified prothrombin with platelet AcG in combination with plasma hemophilic plasma and serum The plasmas and serum were diluted 1 12 in the reaction mixture Temperature 28 C

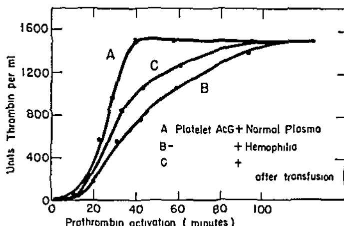


FIG 1 — Activation of purified prothrombin with platelet AcG and combinations of platelet AcG and plasma The hemophilic plasma and normal plasma were both diluted 1 12 in the reaction mixture Temperature 28 C

Actually the incubation tube in the assay contained

(a) Purified Prothrombin (about 3 000 units per ml)	1 00 ml
(b) Platelet AcG	0 50 ml
(c) $CaCl_2$ (0 162M) in imidazole buffer	0 50 ml
(d) The Unknown such as plasma diluted plasma or serum crude preparations pathic plasma etc	1 00 ml

Briefly summarizing this arrangement enables us to measure[19] how much and how rapidly thrombin forms when a mixture of purified prothrombin platelet AcG and calcium ions is tested with unknown materials

Assay of Platelet Co factor in Hemophilic Plasma With diluted hemophilic plasma activation of the purified prothrombin was not as rapid as with an equal amount of similarly treated normal plasma (fig 1 curve A) Eventually an equal amount of thrombin was produced by the hemophilic plasma (fig 1 curve B) and normal plasma The difference between the two is the rate of prothrombin conversion to thrombin This was found without exception in eight cases of hemophilia For proper control purposes it can be seen (fig 1 curve C) that the activation of purified prothrombin in the presence of platelet AcG and calcium ions but in the absence of plasma is very low Calcium and diluted plasma alone also do not activate purified prothrombin rapidly

Normalization of Hemophilic Plasma In the above description a method for preparing the platelet co factor from plasma or serum in crude form is described When the co factor prepared either from serum or plasma is added to hemophilic plasma the activation of purified prothrombin becomes rapid and the preparation from serum seems to have about the same potency as the preparation from plasma

One hemophilic patient 3 years old was given 150 ml of reconstituted dried plasma intravenously Before this the rate of purified prothrombin activation was slow (fig 2 curve B) However after transfusion the rate of prothrombin

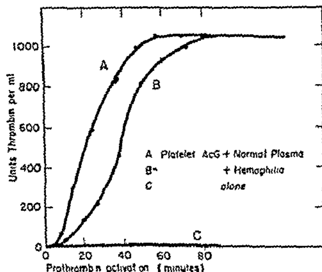


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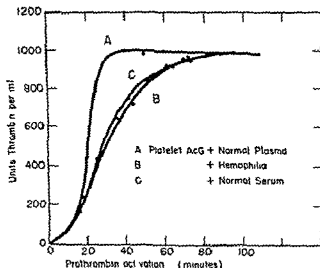


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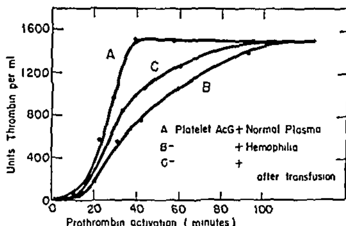


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prothrombin is rapidly and to the same extent a normal human serum The question now would seem to be "Why is hemophilic plasma so similar to normal human serum and why is normal human serum different from normal human plasma under the conditions of our experiment? If we consider the finding of Brinkhous and associates[7] that the antihemophilic factor is not found in hemophilic plasma or in normal human serum then we must consider that it also was not in our hemophilic plasma samples and our normal human serum samples It then follows logically that the hemophilic plasma and normal human serum still contain something which may function in conjunction with platelet AcC to activate the purified prothrombin in the manner described above This component would still need to be considered as a kind of substitute for thromboplastin meaning by thromboplastin that which can be obtained from the fixed tissue such as ground lung and brain extract etc This leads us to the tentative conclusion that normal human plasma contains something which hemophilic plasma does not contain and which normal human serum also does not contain But either one still possesses material comparable to or which can substitute for thromboplastin by functioning in combination with platelet AcG to activate purified prothrombin

Stated in another way The plasma co-factor described in our previous paper may be a composite of at least two substances namely that which is in serum and hemophilic plasma and that which is found in normal plasma

As discussed previously[19] it is becoming more evident that there are many activators of prothrombin and that certain ones can substitute for others or if one is in high concentration it may compensate for deficiency of another Since normal serum is equivalent to hemophilic plasma in our experiments and we can prepare an activator from serum which will correct the hemophilic deficiency in test tubes one is forced to the view that the preparation from serum is a substitute Whether this substitute would function in the veins of a hemophiliac is quite another question

Patton Ware and Seegers[9] were able to prepare essentially platelet free canine plasma by using meticulous care with a silicone technique and found that clotting of such plasma was incomplete there being only a few fibrin strands visible after many hours standing in either silicone or plain glass tubes In similar but more extensive experiments Hartman and associates[1, 20, 4] found that the addition of ground glass to almost platelet free plasma would sometimes cause clotting within 11 minutes often within less than an hour and sometimes within a day Such clotting did not occur when hemophilic plasma was similarly treated Apparently they feel that the glass surface activates a precursor of thromboplastin We do not believe that our work in any way contradicts what was found in their beautiful experiments There are many ways in which prothrombin can become activated as is so plainly evident from the fact that purified prothrombin becomes activated in sodium citrate solutions[4] In the experiments of Hartman and associates probably only very small portions of the available prothrombin became activated as a result of quite incomplete activation of any thromboplastin like material The view that only a small fraction of prothrombin

blood was *collected* by venipuncture from healthy individuals and allowed to coagulate and stand for about two hours at room temperature. The resulting serum was centrifuged in a refrigerated centrifuge for 30 minutes at 1,500 g. The serum was then diluted two fold with saline because it was desired to have the sample comparable to plasma samples which were diluted in defibrination. The serum was then extracted three times with equal volumes of ether, to remove antithrombin and stored in the deep freeze.

When assayed for platelet co factor by the method described above the serum (fig. 3, curve C) was found to activate purified prothrombin at almost exactly the same rate as hemophilic plasma (fig. 3, curve B).

Discussion

Apart from vascular factors there are two important theories of the cause of the dysfunction of hemostasis in hemophilia. One is that the plasma is deficient in a platelet co factor. With less platelet co factor, less thromboplastin equivalent is formed by the interaction of platelet co factor and platelets and the rate of activation of prothrombin is less. The other view is concerned with the fact that hemophilic plasma contains more of the inhibitor, antithromboplastin, than normal plasma. As a result the action of thromboplastin is considered to be inhibited. In our experiments the effect of antithromboplastin was essentially ruled out and thus they do not contribute directly to our knowledge about this factor in hemophilia. We believe it was not concerned in our experiments because the technique of assay for platelet co factor involves dilution, ether treatment of serum and plasma, and storage in the deep freeze. Any one of these manipulations is considered to destroy antithromboplastin.[3]

It has been indicated that the activators of purified prothrombin are numerous and their anatomic location may be considered to be in the fixed tissue, in the platelets and in the plasma. By using the material in these compartments it is possible to prepare activators in such a way that any combination of two of these compartments will produce rapid activation of purified prothrombin. Thus any one of the anatomic compartments can be completely abandoned in test tube experiments. When the plasma compartment functions in combination with the platelet compartment in the rapid activation of purified prothrombin, the fixed tissue compartment is abandoned; hence the substance commonly referred to by the term thromboplastin is abandoned and a substitute has been furnished by the combination of platelets and plasma material.

In a previous contribution from this laboratory the plasma material was referred to by the term platelet co factor because it functioned in combination with platelets and more particularly with the platelet fraction containing platelet accelerator or platelet AcG.[11] In the experiments described above we found that the plasma of hemophiliac individuals does not function as effectively in combination with platelet AcG or platelet extract as normal human plasma does. It was, however, possible to activate purified prothrombin with relative rapidity and to obtain a yield of thrombin comparable to that which could be obtained by the use of thromboplastin. The hemophilic plasma activated the purified

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was activated might have been substantiated if prothrombin utilization experiments had been done in their study In our experiments, diluted plasma alone caused very little activation of purified prothrombin likewise platelet extracts alone caused very little activation of purified prothrombin but the combination of the two caused rapid activation To be sure, measurable amounts of thrombin were always produced with either factor alone but the contrast which resulted from a combination of the two is so great as to lead one practically to disregard the minute amounts formed slowly by either platelet extract alone or the plasma factor alone

Summary

Activators of purified prothrombin are many and are found to substitute for one another In hemophilia a platelet co factor normally found in plasma is apparently lacking This co factor acts in conjunction with platelets to furnish the equivalent of lung extract thromboplastin in the activation of purified prothrombin

Hemophilic plasma normal human serum and hemophilic serum also contain platelet co factor activity That is the equivalent of lung extract thromboplastin is produced when platelets are mixed with normal human serum hemophilic serum or hemophilic plasma Since normal serum hemophilic serum and hemophilic plasma contain equal amounts of platelet co factor activity and since this is less than is found in normal plasma it is postulated that normal plasma contains two platelet co factors One of these disappears when blood clots and is not found in serum and this same one probably is absent in hemophilic plasma normal serum or hemophilic serum

Our assay technique for platelet co factor may be valuable in the diagnosis of hemophilia and the classification of hemorrhagic diseases

ACTIVACION DE PROTROMBINA PURIFICADA CON PLASMA HEMOFILICO

Los activadores de la protrombina purificada son varios y son capaces de substituirse unos por otro En la hemofilia un co factor de plaquetas que se encuentra normalmente en el plasma se halla ausente Este co factor en conjuncion con las plaquetas actua proveyendo el equivalente de la tromboplastina extraída del pulmon en la activacion de la protrombina purificada

El plasma hemofílico el suero humano normal y el suero hemofílico también contienen actividad debida al co factor plaquetario Se deduce de esto que el equivalente de tromboplastina extraída de tejido pulmonar se produce cuando se mezclan las plaquetas con suero humano normal suero hemofílico o plasma hemofílico Como el suero normal el suero hemofílico y el plasma hemofílico contienen iguales cantidades de co factor de las plaquetas y como ésta es menor que la que se encuentra en plasma normal podemos postular que el plasma normal contiene dos co factores de las plaquetas Uno desaparece cuando la sangre se coagula y no puede ser hallado en el suero siendo probablemente este mismo el que se halla ausente en el plasma hemofílico suero normal o suero hemofílico

La investigacion de este co factor de las plaquetas puede ser de gran valor en el diagnostico de la hemofilia y en la investigacion de las enfermedades hemorragíparas

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tions of a number of cases, in which variations of factor VII activity were observed included thromboembolic diseases pregnancy (increase of factor VII) newborn liver diseases treatment with Dicumarol and allied compounds (decrease of factor VII) In this last condition it has been shown that factor VII preparations obtained from animals treated with Dicumarol and allied compounds have a less pronounced factor VII activity as compared with preparations from normal animals Furthermore the determination of factor VII activity in various species in connection with other blood clotting variables allowed us to correlate it rather closely with the values of prothrombin time than with the prothrombin and Ac globulin values

Methods and Material

The following methods have been used two stage method for the determination of the prothrombin and Ac globulin concentration[11, 20] one stage method for factor VII quantitative determination[11] one stage method for the determination of the prothrombin time[21] The percentages have been obtained from the times in the one stage methods by using standard curves as described in previous reports[11, 21, 6] In the two stage methods the *percentages have been evaluated by taking as 100% the mean values for human beings and* by calculating accordingly the other percentages

Preparations of factor VII were obtained by using a recently described method[22] The preparations were frozen or dried from the frozen state and then redissolved in distilled water The method is based on the absorption of the factor from plasma or from serum by means of barium sulphate and successive elution with sodium citrate The various phases of the preparations followed exactly the method mentioned above

Dicumarol and Tromexan were administered to dogs at the dose level of 10 mg /kg /day (Dicumarol) or 50 mg /kg /day (Tromexan) for four days The animals were then exsanguinated for the collection of oxalated plasma (9 parts blood and 1 part sodium oxalate 1 34%) and serum

The animals used in the species experiments included 4 rats 4 cats 12 humans 4 specimens of pooled cow plasma 4 dogs 4 guinea pigs 4 rabbits The blood was obtained by heart or by venous puncture and mixed with sodium oxalate 1 34% in the proportions 9 parts blood and 1 part oxalate The clinical material included 12 normal subjects 22 cases of late normal pregnancy 47 cases of thromboembolic diseases 31 cases of liver cirrhosis 61 cases of other liver diseases 22 cases of newborn without manifest bleeding tendency

The results are reported in the graphs and represent the percentages obtained in the various experiments or in the cases of the species experiments the mean values of the calculated percentages

The thromboplastin used in all these determinations was the product RO 1-44 6/250 kindly supplied by Hoffmann LaRoche A G Basel Switzerland through the courtesy of Hoffmann LaRoche Inc Nutley N J

Results and Discussion

Factor VII activity in various physiopathological conditions A number of physiopathological conditions allowed us to observe variations of factor VII activity (fig 1) The *increase in activity of factor VII represents a particularly* interesting field insofar as the methods for detecting a hypercoagulability tendency are not numerous and not all reliable In late normal pregnancy the increase of fibrinogen and of platelets were correlated with the hypercoagulability tendency (see details in Baserga and de Nicola[4, 5]) Further research brought up the concept of a hyperprothrombinemia[4, 73] not accepted however by all

VII 5

Studies on the Mechanism of Factor VII Activity

PIETRO DE NICOLA*

THE ATTENTION of investigators of blood coagulation phenomena has been recently focused on new clotting factors included during the last ten years but which were missing in the older coagulation schemes It has been tentatively suggested that at least three provisional groups of factors be made beside calcium, which are involved in the conversion of prothrombin into thrombin [1]

A first group includes the factors concerned with the formation of thromboplastin Recent research carried out with the technique of the purified systems has established that a plasma and a platelet factor are involved in this phenomenon [2]

The other two groups include the so called accelerating and converting factors The former includes factors which have been already extensively investigated such as plasma and serum Ac globulin,[3, 13] factor V and VI [4] labile factor,[5] accelerator globulin of Fantl and Nance [6] plasma co factor of thromboplastin [7] plasma prothrombin conversion factor (PPCF) [8] proaccelerin and accelerin [9] etc The latter concerns factors recently described such as the observations referring to serum prothrombin conversion accelerator (SPCA) and its precursor [10] proconvertin convertin [9] factor VII [11] co thromboplastin [12] etc

The terms of accelerating and converting factors are conventional and are derived from some factors included in these groups It seems that the so called accelerating factors increase not only the speed of activation of prothrombin but also the amount of prothrombin converted into thrombin [13] Conversely the so called converting factors like factor VII are supposed to affect not the thrombin yield but only the rapidity of prothrombin conversion [11]

It is known that marked deficiencies of these groups of factors may produce a coagulation defect resulting in hemorrhagic diathesis [4, 14, 19] The congenital forms seem to represent the most significant observations, insofar as they allowed us to identify with more certainty the patterns of the specific defect

The availability of a technique for the "quantitative" determination of one of the so called converting factors such as factor VII [11] made it possible to investigate its physiopathological significance in various experimental and clinical conditions In the research presented in this paper it has been attempted to further characterize the behaviour of this new clotting factor The clinical observa

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Some of the research presented in this work was carried out at the Department of Physiology of Wayne University during the tenure of a U S Government Fellowship administered by the Institute of International Education New York N Y

Concerning the coagulation defect in the liver diseases and in the newborn the finding of a decreased factor VII activity is in keeping with the previous findings of a decreased prothrombin and Ac globulin concentration (see details[2]) It has been stated that in liver diseases the variations of factor VII are more pronounced than the ones of prothrombin[24] When hypoprothrombinemias in liver diseases are sensitive to vitamin K also factor VII shows significant increase [28, 33]

Factor VII variations during treatment with Dicumarol and allied compounds It has been observed that factor VII decreases in concentration when Dicumarol[11, 25] or other related compounds [4, 40] are administered It has not been tried however to see whether less active factor VII preparations are obtained from Dicumarol plasma than from normal plasma when those respective plasmas are used as starting material for the preparation of factor VII Simple technics have been devised for obtaining factor VII in concentrated form[22] If factor VII activity is not found in Dicumarol plasma in appreciable quantities the chemical technics for obtaining concentrations should yield less from the pathic plasma The lowered yield might be interpreted as indicating that there was decreased factor VII activity in the first place and considerable confidence could be placed in such an experiment even though it cannot be conclusive In our experiments the activity of the preparations obtained from treated animals was less pronounced than the one of preparations obtained from normal animals Such preparations were also obtained in dry form By dissolving the same

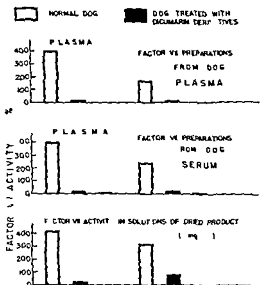


Fig 2 – Above and in the middle Factor VII activity in the plasma (left) and in factor VII preparations (right) obtained from normal dogs and from dogs treated with Dicumarol (preparations from plasma) or Tromexan (preparations from serum) Below Factor VII activity in preparations containing 1 mg dry product per cc obtained from normal dogs and from dogs treated with Tromexan

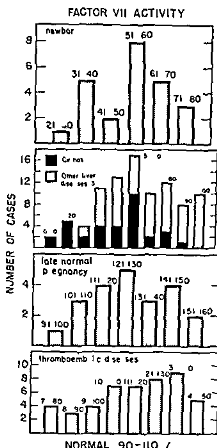


FIG 1 — Factor VII values in various physiopathological conditions increased factor VII activity in thromboembolic diseases and in late normal pregnancy (9th month) decreased factor VII activity in liver diseases and in the newborn

authors [29, 30] We have determined that there is an increase above normal in the amount of factor VII in the blood of pregnant women at or near term The prothrombin concentration may be normal or even decreased as we could observe in some cases even when the factor VII activity was above the normal level *

The same is true also for thromboembolic diseases in which a significant increase of factor VII was observed in several cases In these forms numerous attempts had been made especially during the last years to characterize the coagulation tendency the whole blood clotting time in glass and silicone [31, 32] the heparin tolerance test in vitro and in vivo [33, 34] the Ac globulin determinations [35, 36] the fibrinogen concentration [36] and the prothrombin time in diluted plasma [37] Factor VII determinations seem to be suitable for this purpose because they may reflect the hypercoagulability tendency even when other factors are occasionally decreased and therefore not able to reveal such a tendency (fig 1)

* The data concerning factor VII variations in pregnancy were obtained through the cooperation of Dr A Iovotti Dept of Obstetrics and Gynecology University of Pavia

Our results seem to offer further insight into the discrepancies of a different factor VII activity In the example given above (bovine plasma), in spite of the high Ac globulin concentration the prothrombin activity as measured by the one stage method is low in agreement with the low factor VII activity High concentrations of Ac globulin are not able to give a short prothrombin time when the factor VII activity is not very high (fig 3) Furthermore these data support the concept that the prothrombin time should essentially reflect at least in normal conditions the activity of factors like factor VII We know how ever, that in the presence of an isolated deficiency of factor V or Ac globulin the prothrombin time is increased even though the concentration of the other factors is normal

Conclusions

The physiopathological significance of factor VII in the blood coagulation mechanisms can be further explained on the basis of the previously reported results A first remark may be made in connection with the finding of a decreased factor VII activity in various conditions In all forms in which a decrease of factor VII was found also prothrombin was decreased The only exception seems to be represented by the congenital specific defects of factor VII or allied factors without alterations of the other factors If one tries to bring up to normal levels the decreased factor VII activity also prothrombin follows the same patterns This is true for liver diseases for the treatment with Dicumarol and allied compounds for the coagulation defect of the newborn The almost constant association of the factor VII deficiency with prothrombin deficiencies emphasizes the fact that it is difficult to separate the e two factors by means of various procedures (Seitz filtration adsorption) Some properties of factor VII and prothrombin seem to be therefore similar and this fact could explain why their variations almost always parallel at least in terms of the employed methods

Concerning the increased factor VII activity the data arising from the experiments in various species in thromboembolic diseases and in pregnancy are suggesting the possibility that factor VII be correlated with the rapidity of prothrombin activation at least in the considered experimental conditions The rate of blood coagulation has some significance for the detection of a coagulation tendency and the consequent therapeutic directions The factor VII determination might be used extensively to give definite information about such a problem The diagnosis of a thrombosing disease and the administration of anticoagulants may be discarded on the basis of other tests while the identification of an increased factor VII concentration gives the necessary support to such implications

Furthermore the observations concerning the recovery of factor VII from normal and treated dogs represent the application to this new factor of procedures already employed for prothrombin In animals treated with Dicumarol and allied compounds also prothrombin has been recovered in a quantitative way and the yield has been smaller than in normal animals[44] In our experiments it might be possible that the variations observed be only concerned with

amounts of these dry preparations in the same amount of liquid, the same kind of differences were observed (fig 2)

Factor VII activity in various species The behavior of factor VII in various animals has been compared with other variables such as prothrombin time as measured by the one stage method, Ac globulin and prothrombin concentration (two stage method) Such investigations are of interest not only for the field of comparative physiology but also for the further elucidation of the mechanisms of factor VII activity The statement that the original one stage method for the prothrombin determination not only refers to prothrombin but also to other factors had already been made when the observations about the new factors were not yet extensively known [41, 42] However the discovery of a first group of new factors such as Ac globulin made it possible to establish that the prothrombin time as measured by the one stage method was a composite effect depending at least on the concentration of prothrombin and this new factor when the other variables were kept constant The identification of another group of new factors such as the so called converting factors suggested the possibility that such factors might also be involved in the results of the prothrombin time

By determining factor VII activity in various mammals it was possible to observe that its behaviour follows very closely the variations of the prothrombin time and is not correlated with the variations of the prothrombin and Ac globulin concentrations Early studies on the behaviour of the clotting factors in the various species attempted to determine whether the discrepancies between the one and two stage methods for prothrombin determination could be on the basis of Ac globulin variations This was found not to be the case For instance in the case of bovine plasma it was not quite understandable why a prolonged prothrombin time should correspond to a normal prothrombin concentration in the two stage method and to an extremely high Ac globulin concentration [43]

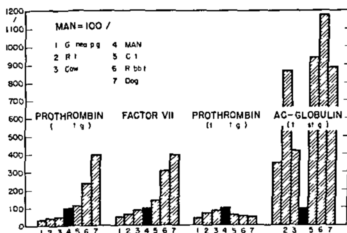


FIG 3—Factor VII Prothrombin (one stage) Prothrombin (two stage) Ac Globulin (two stage) determinations in various mammals correlation between factor VII and prothrombin (one stage) values no correlation between prothrombin (one stage) and prothrombin (two stage) or Ac Globulin (two stage)

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VII 6

An Unusual Form of Hemophilia in Humans

JOHN BORDEN GRAHAM*

A MILD hemorrhagic disease was discovered in a large American family of English and German extraction. Symptoms were limited to 14 of the males in the family. Clinically, the disorder in these men was characterized by nosebleeds, excessive bleeding after tooth extraction or other surgery and easy bruising. No history or evidence of hemarthrosis was present, and there had

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factor VII activity as measured by the 'quantitative' method. As the methods for factor VII preparation are not yet as refined as the methods for the preparation of other coagulation factors, we do not rule out the possibility that other factors could be implied with such preparations. In fact it has been found that in the barium sulphate eluate substances are present which have different properties from factor VII and allied factors.[45]

Summary

1. Increased factor VII activity has been observed in late normal pregnancy and in thromboembolic diseases.

2. Decreased factor VII activity has been found in liver diseases and in the newborn.

3. Factor VII preparations obtained from animals treated with Dicumarol and allied compounds have a less pronounced factor VII activity than preparations from normal animals.

4. The comparison of factor VII activity in various species showed a parallel behaviour between factor VII activity and prothrombin time but not with prothrombin and Ac globulin concentrations.

5. It was suggested that factor VII should be concerned with the rate of blood coagulation either in physiological or in pathological conditions.

Estudios sobre el Mecanismo de la Actividad del Factor VII

1. La actividad del factor VII se halla aumentada en los ultimos estadíos del embarazo normal y en las enfermedades tromboembólicas.

2. La actividad disminuida del factor VII ha sido hallada en afecciones hepáticas y en el recién nacido.

3. Preparaciones del factor VII obtenidas de animales tratados con Dicumarol y drogas análogas tienen una actividad menor que la que se encuentra en preparaciones de animales normales.

4. El estudio comparativo de la actividad del factor VII en varias especies mostro un compartamiento paralelo con el tiempo de protrombina pero no con las concentraciones de protrombina y globulina Ac.

5. Se ha sugerido que el factor VII estaría relacionado con la coagulación sanguínea ya sea en condiciones normales o patológicas.

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VII 6

An Unusual Form of Hemophilia in Humans

JOHN BORDEN GRAHAM*

A MILD hemorrhagic disease was discovered in a large American family of English and German extraction. Symptoms were limited to 14 of the males in the family. Clinically the disorder in these men was characterized by nosebleeds, excessive bleeding after tooth extraction or other surgery, and easy bruising. No history or evidence of hemarthrosis was present, and there had

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never been a death from hemorrhage The affected males have lived active athletic lives essentially unhampered by this hemorrhagic tendency

The mean clotting times and prothrombin utilization rates (one and two stage types) were slightly but significantly slowed in the affected men However, the wide range of variation with these tests rendered them of little value in individual cases Platelet counts bleeding times, capillary fragility and anti thrombin levels were normal No circulating anticoagulants were detected Accelerator globulin levels were normal and serum accelerator(s) evolved normally during clotting

The only clotting test which was consistently abnormal in these men was the test for the antihemophilic factor (AHF) the clotting factor which is low or inactive in hemophilia Two assays for this factor have been developed in our laboratory and both were used in studying this family The original assay (Proc Soc Exp Biol & Med , 77 294 1951) takes advantage of the fact that the greatly slowed prothrombin consumption in hemophilic blood can be increased almost to normal by addition of normal plasma AHF activity of a test plasma can be compared with that of a control plasma by determining the dilution of each which increase prothrombin utilization in hemophilic blood to the same degree The AHF level of a test plasma is expressed in percentiles of the normal control The newer assay procedure (J Lab & Clin Med , in press) is a greatly simplified one based on the fact that the clotting time of hemophilic plasma is longer than that of normal plasma when both are accelerated with cephalin a partial thromboplastin When normal plasma is mixed with hemophilic plasma the cephalin clotting time of the mixture reache that of a wholly normal plasma when about 15–20 per cent of the mixed plasma is the normal type

With these assay procedures it was found that the affected men had AHF levels ranging from 9 to 25 per cent of normal by the simplified procedure (12 to 22 per cent by the original procedure) The range for 28 normal adults was 68 to 165 per cent of the mean (100 per cent) The plasma of classical hemophiliacs showed at most less than 2 per cent AHF activity Thus it appeared that this mild hemorrhagic disease whose only distinct abnormality was reduced AHF activity was quite different from the usual form of hemophilia

Interestingly about one half of the heterozygous females (mothers of affected males) showed a reduced AHF level The reduction in these women was less than in the affected men the mean level being 42 per cent The other heterozygous women had normal AHF activity The low AHF level of some of these heterozygotes emphasized another difference between this mild disease and classical hemophilia Merskey in human heterozygotes and ourselves in dogs have been unable to detect a lowered AHF level Recently we tested 3 mothers and 3 sisters of hemophiliacs and found all AHF levels in the normal range 68 to 128 per cent

Studies of the pattern of inheritance suggested a sex linked recessive type of transmission Statistical studies showed that the hypothesis of sex linked recessive mode of inheritance could not be rejected The probability was very slight that autosomal dominant or autosomal recessive inheritance had occurred

Thus the weight of evidence is that this family has hemophilia in a mild form distinctly different from the usual form. It has frequently been observed that both mild and severe hemophilia occur in a family transmitting the classical hemophilia gene. In this family the disease has been mild in all 14 affected males.

The differences between this mild form of hemophilia and the classical form have led us to postulate that this family has an allelic form of hemophilia (Am J Med Sci 225 46 1953). Thus if the dominant normal gene is designated *H* and the recessive gene for classic hemophilia *h* we propose to designate the gene carried in this family as h^m. The *h* is to indicate probable recessivity and the superscript *m* to indicate that the gene is responsible for a mild form of the disease. It is not entirely clear that the h^m mutant is the complete recessive that the *h* mutant appears to be. The mild hemophilia gene might be a partially dominant gene lacking complete penetrance.

The action of the postulated hemophilia alleles appears complex. The classic gene *h* causes a severe disease when present alone in males. When present in females as *Hh* however it has no obvious effect. The mild gene h^m in males has a mild effect but half the time has a more severe effect as Hh^m than does the classic heterozygous combination *Hh*. It would be of great theoretical interest to study the hh^m combination. It is already known that females of the genotype *hh* both in man and dog have a severe form of hemophilia indistinguishable from the classic hemophilia of males.

For the present we are postulating the existence of an allelomorph series occupying the hemophilia locus on the X chromosomes. There appear to be at least 3 alleles in this series but there may be others. The fact that AHF levels vary widely among normal humans and only slightly in an inbred strain of normal dogs suggests that there may be several dominant alleles in humans (H H H′ etc.) each responsible for a characteristic AHF level. Thus the differing effects of the same hemophilia mutant h^m in heterozygous females may be due to the particular normal allele which forms its mate in the gene pair.

The failure to recognize this form of hemophilia previously is probably due to the mild effect of the gene and the lack of tests sensitive enough to diagnose it accurately. The availability of simple AHF assays makes it possible now to classify many of the unclassified "bleeders" in the population and establish the density of the hemophilia genes.

Forma Atipica de Hemofilia en el Hombre

Este trabajo se refiere a una enfermedad hemorragípara benigna descubierta en una numerosa familia americana de ascendencia inglesa y alemana. Los síntomas se limitaron a 14 de los varones de esta familia. Clínicamente se caracterizaron por hemorragias por epístaxis, hemorragias excesivas post-extracciones dentarias u otras formas de cirugía y tendencia a las equímosis. No había antecedentes de hemartrosis ni fallecimientos por hemorragias. Los varones afectados han llevado una vida activa, atletica y poco alterada por esta tendencia hemorrágica.

El único defecto de coagulación persistente en estos sujetos fué el test del factor antihemofílico (AHF), factor que se encuentra reducido o inactivo en la hemofilia.

Se encontró que los niveles de AHF en los casos que se describen variaron de 9 a 25% de la cifra normal (segun el método simplificado descripto por el autor). La cifra hallada en 28 adultos normales varió entre 68 y 165%. El plasma de las hemofilias clásicas mostro menos

del 2% de la actividad del AHI Por lo tanto esta enfermedad hemorragica benigna cuya unica anormalidad fue una reduccion de la actividad del AHF era distinta de la forma usual de hemofilia

Como dato interesante se encontró que aproximadamente un 50% de las mujeres hétero cigotas madres de los varones afectados mostraron un nivel de AHF reducido Esta reduccion fue menor que en los varones afectados siendo el nivel promedio de 42% El resto de las mujeres héterocigotas mostró una actividad normal de AHI El nivel bajo del AHF de algunas de esta mujeres muestra otra diferencia entre esta enfermedad benigna y la hemofilia clásica

El estudio hereditario de esta familia sugiere que la transmisión se haria por medio de un carácter sexual recesivo

De los estudios efectuados se deduce que esta familia tiene una hemofilia leve distinta de la forma usual Las diferencias entre ambas formas nos han inducido a postular que esta familia tendria una forma alelomorfa de hemofilia Si denominamos al gen dominante normal *H* y al gen recesivo para la hemofilia clásica *h* proponemos designar al gen transportado en esta familia *hm* La h es para indicar la probabl recesividad y la *m* indica la forma leve de la enfermedad

Como resultado de estos estudios postulamos la existencia de una serie alelomorfica que ocupa el locus hemofilico en los cromosomas X

La dificultad de reconocer esta forma de hemofilia en la anterioridad se debe probablemente al leve efecto del gen y a la falta de tests suficientemente sensibles para su diagnóstico La existencia del test para medir el AHF hace posible hoy dia la clasificacion de muchos de los sangradores no clasificados y servirá para establecer la densidad de los genes hemofilico en la poblacion

VII 7

La Técnica de Quick con Tromboplastina Calentada a 60 en la Hemofilia y Otros Síndromes Hemorragiparos

M DERECHIN M KLEIMANS y N R PERRONE*

PREOCUPADOS ante la necesidad de una técnica de laboratorio que permitiese el diagnóstico de certeza de hemofilia hemos acogido con interes la tecnica de Quick Stapp y Hussey con tromboplastina calentada a 60 y la hemos usado con los pacientes hemofilicos y purpuras trombocitopenicas del Instituto Municipal de Hematologia Policlinico Ramos Mejía Sala XVIII

En este trabajo hemos respetado estrictamente la tecnica original excepto en el tiempo esperado para hacer la prueba final con plasma nativo Tenemos nosotros la preocupacion rutinaria de investigar la presencia de trombina en todos los sueros usados para las pruebas de consumo de la protrombina y habiendo encontrado vestigios de trombina activa en plazos menores decidimos esperar 30 minutos en lugar de 15

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TABLA 1.—*Hemofilia*
(Cifras en segundos. Lee White en minutos)

Paciente	P.N.Pb.Pq.	P.N.R.Pq.	P.N.Pb.Pq. + Tp. 60	P.N.Pb.Pq. + Tp. 50	Tiempo de tromboplastinogenio	Lee White
1	8	9	9½	165	8½	90
2	7½	9½	10	75	12	15
3	8	8	8	170	9½	150
4	8	11	9	67	10	16
5	8	10	11	31	11	125
6	8	8	8	15	—	12
7	8	9½	8½	91	11	30

P.N.Pbr.Pq.: Plasma nativo pobre en plaquetas.
P.N.R.Pq.: Plasma nativo rico en plaquetas.

Los resultados obtenidos son coincidentes con los de Quick y col., según puede verse en las tablas siguientes.

En la tabla 1 puede verse que el agregado de tromboplastina calentada a 60° no normaliza el consumo de la protrombina en los pacientes hemofílicos.

En la tabla 2 puede verse que el agregado de tromboplastina calentada a 60° normaliza el consumo de la protrombina de los pacientes trombocitopénicos.

En la tabla 3 puede verse que los pacientes normales aumentan el consumo de la protrombina con el agregado de tromboplastina.

La tabla 4 reúne, a modo de resumen y para hacer resaltar más las diferencias, los ejemplos más salientes de las tablas anteriores.

La síntesis gráfica reúne en tres curvas el promedio de los resultados obtenidos con los plasmas nativos. Puede verse una curva correspondiente a los pacientes normales, con sólo el primer tubo debajo del límite de normalidad; una curva trombocitopénica, con los tubos 1 y 2 por debajo del límite de normalidad, y la curva de los pacientes hemofílicos, que presentan los tres primeros tubos con promedios subnormales.

No es nuestro objeto discutir las consecuencias teóricas del nuevo método, pero en relación a su valor práctico, creemos, de acuerdo con nuestros resultados,

TABLA 2.—*Púrpura Trombocitopénica*
(cifras en segundos)
(Menos de 30.000 plaquetas/mm³ en todos)

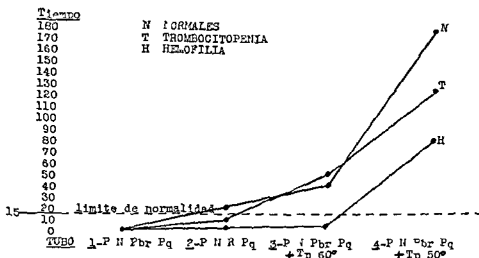
Paciente	P.N.Pb.Pq.	P.N.R.Pq.	P.N.Pb.Pq. +Tp. 60	P.N.Pb.Pq. +Tp. 50	Tiempo de tromboplastinogenio
8	9	10½	15	143	18
9	8	10	71	140	65
10	8½	10½	60	120	49
11	7½	11	49	110	51
Trombopatía Constitucional (300.000 plaquetas/mm³ sin aglutinación)					
12	8	23	40	110	88

TABLA 3.—*Normales*
(cifras en segundos)

Paciente	P N Pbr Pq	P N R Pq	P.N.Pbr Pq +Tp 60	P.N.Pbr Pq +Tp 50	Tiempo de tromboplastinógeno
13	8	17	50	300	99
14	9	15	22	37	74
15	9½	18	21	153	78
16	9½	16½	54	225	123

TABLA 4.—*Síntesis Numérica*

Paciente	P N Pbr Pq	P N R Pq	P N Pbr Pq +Tp 60	P N Pbr Pq +Tp 50	Tiempo de tromboplastinógeno
3	8	8	8	120	91
5	8	10	11	31	11
9	8	10	71	140	65
13	8	17	50	300	99



SÍNTESIS GRÁFICA (promedio de cada uno de las pruebas con plasma nativo)

que es por sí solo capaz de darnos el diagnóstico de hemofilia y por lo tanto el diagnóstico diferencial con otros síndromes hemorragíparos acompañados de bajo consumo de la protrombina.

QUICK'S METHOD WITH THROMBOPLASTINE HEATED AT 60° IN HEMOPHILIA AND OTHER HEMORRHAGIC SYNDROMES

Concerned with the necessity of a safe laboratory technique which would permit a sure diagnosis of hemophilia, we have applied Quick's technique on our patients, obtaining results which agree with those obtained by the authors and can be seen on the Tables in the following order:

1. Natural plasma with subnormal quantity of platelets (thrombocytopenia).
2. Natural plasma with abundant quantity of platelets (thrombocytosis).

3 Natural plasma with subnormal quantity of platelets and 60 thromboplastine
4 Natural plasma with subnormal quantity of platelets and 30 *thromboplastine*
5 Thromboplastinogenous time

We also show a diagram with the results obtained with natural plasma upon which can be seen three types of curves the normal one with only the first tube below the normal limit the thrombocytopenic one with the first and second tube below normal limit and the hemophilic one with the first three tubes below normal

We have strictly respected the original technique in our investigation save what concerns the time interval for the test with natural plasma On effecting the prothrombin consumption tests we have the routine precaution of controlling the total inactivation of thrombin and having found traces in certain cases where we only waited fifteen minutes we prolonged our waiting time to 30 minutes

No pretense is made in discussing the theoretical consequences of *the new method* but with respect to its practical value it seems probable to us according to *our results that this* is a method capable of giving by itself a sure diagnosis of hemophilia consequently it may be used for the differential diagnosis of other hemorrhagiparous syndromes accompanied by low consumption of prothrombin

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VII 8

Hematologic Effects of Poly-DL-lysine in Vitro and in Vivo

A DE VRIES, Y STEIN O STEIN, J FELDMAN
J GUREVICH and E KATCHALSKI*

SYNTHETIC poly a amino acids are straight chains of amino acids bound in peptide linkage Poly lysine with free amino-groups is a basic poly amino acid (fig 1)

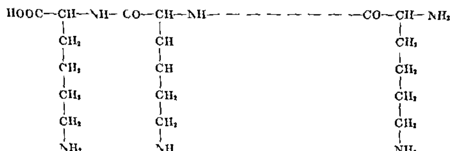


Fig 1 —Poly lysine

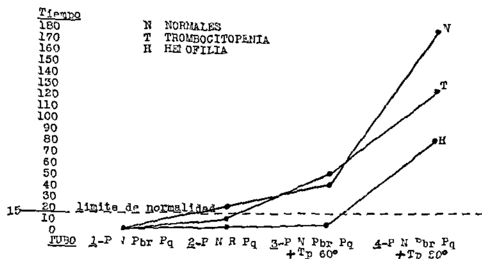
* Rothschild Hadassah University Hospital Jerusalem and Weizmann Institute for Science Rehovoth Israel

TABLA 3 —*Normales*
(cifras en segundos)

Paciente	P N Pbr Pq	P N R Pq	P N Pbr Pq		Tiempo de tromboplastinógeno
			+Tp 60	+Tp 50	
13	8	17	50	300	99
14	9	15	22	37	74
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Paciente	P N Pbr Pq	P N R Pq	P N Pbr Pq		Tiempo de tromboplastinógeno
			+Tp 60	+Tp 50	
3	8	8	8	[illegible]	91
5	8	10	11	31	11
9	8	10	71	140	65
13	8	17	50	300	99



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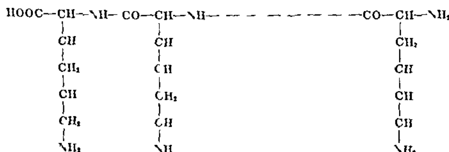


Fig 1 —Poly lysine

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Poly L aspartic acid with free carboxyl groups is an acidic poly amino acid (fig 2)

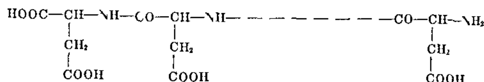


FIG 2 —Poly aspartic acid

Poly alanine with free methyl groups is a neutral poly amino acid (fig 3)

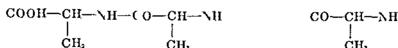


FIG 3 —Poly alanine

It was previously reported that poly lysine with a chain length of 35 amino acid units has strong anticoagulant and antiheparinic activity It was also shown that poly lysine disturbs the formation of thrombin manifested by the elevation of serum prothrombin This disturbance of thrombin formation was ascribed to an antithromboplastic activity of poly lysine

Poly aspartic acid does not prolong clotting time but is capable of neutralizing the anticoagulant effect of poly lysine Polyalanine has no anticoagulant or antiheparinic activity nor does it neutralize the action of poly lysine

We are reporting here the effects of intravenously administered poly DL lysine on the blood coagulation of rats Solutions of poly DL lysine hydrochloride in isotonic saline were slowly injected into a rat tail vein in a total quantity not exceeding 0 4 ml Blood was obtained at various intervals by puncture of the heart exposed under ether anesthesia avoiding admixture with tissue juice The clotting times were measured at 37 C Prothrombin of the serum obtained one hour after clotting was estimated by the method of Rosenfield and Tuft using human $BaSO_4$ treated oxalated plasma as diluent and human brain thromboplastin extract Rat serum prothrombin values are expressed in percentage of human plasma prothrombin The plasma prothrombin of the rats varied from 130–190% (table 1)

Rats given lethal doses of poly lysine (1 5 mg or more per 100 Gm body weight) died with pulmonary edema and neurologic disturbances but without hemorrhagic manifestations The lethal dose of 8 mg poly lysine prolonged the clotting time markedly Lethal doses of 1 5–2 mg did not affect the clotting time nor did smaller nonlethal doses However a definite disturbance in thrombin formation as indicated by elevated serum prothrombin was observed even with doses as low as 0 05 mg per 100 Gm body weight It was found that the anticoagulant effect of intravenously administered poly lysine remained detectable up to 2–3 hours after injection and had disappeared within 6 hours

Heparin synthetic poly L aspartic acid and natural poly D glutamic acid protected rats from the fatal effects of intravenous poly lysine either by mixing

TABLE 1—*Effect of intravenous poly DL lysine HCl on blood coagulation of rats*

Poly lysine HCl mgm per 100 Gm body weight		Clotting time min	Serum prothrombin	Number of experiments
—		1 5–5 5	0	5
8	†2 minutes	33	50	1
?	†6 10 minutes	0–? 5	1 0–190	3
1 5	†10 minutes or recovery	1 5–3 5	100 180	3
1 0	Dyspnea	1 0–4 0	85–100	2
0 5	Dyspnea	3 0–3 5	50–52	2
0 2		3	43 110	2
0 05		2	0–57	2
0 025 and lower		1 5–2 5	0	3

Blood taken 10 minutes after injection of poly lysine

poly lysine with it before injection or by intravenous administration not more than 3 minutes after injection of poly lysine In only a few of several experiments however did the acidic polymers neutralize completely the disturbance in thrombin formation caused by poly lysine The clot retarding effect of heparin in vivo could be neutralized by poly lysine either by mixing with heparin before injection or by intravenous administration a short time after the injection of heparin Effective acidic polymer to basic polymer ratios are 1 1 or 1 5 1

A second phenomenon to be reported is the agglutination of red cells by poly lysine in vitro Poly L aspartic acid and poly alanine do not agglutinate red cells (fig 4)

The lowest concentration of poly lysine required to cause agglutination in a 2% saline suspension of washed human or rat red cells is 5–10γ per ml The amount of poly lysine required to agglutinate red cells in the presence of plasma proteins is much larger In order to agglutinate red cells in human or rat whole blood 250γ or more of poly lysine per ml is required The blood of rats injected intravenously with lethal doses of 1 5–2 5 mg of poly lysine per 100 Gm body weight does not show red cell agglutination presumably because of the binding of the poly amino acid to the plasma proteins However in the heart blood of the rat dying after injection of 8 mgm of poly lysine intravenously red cell agglutination was observed

A third phenomenon to be discussed is the hemolytic effect of poly lysine Washed human red cells suspended in isotonic saline glucose or sucrose buffered at pH 7 3 hemolyzed rapidly in the presence of poly lysine the rate of lysis increasing with rising concentration of poly lysine (fig 5)

In the presence of 500γ poly lysine per ml a 3% washed red cell saline suspension liberated within 60 minutes at 37 C 50% of the red cell hemoglobin in excess of the control without poly lysine Phase microscopic observation of the red cells in the presence of poly lysine at pH 7 3 in isotonic saline glucose or sucrose showed the appearance of dark granules at the edges of the ghosts which stained black with Sudan Black B and red with Sudan IV It is postulated

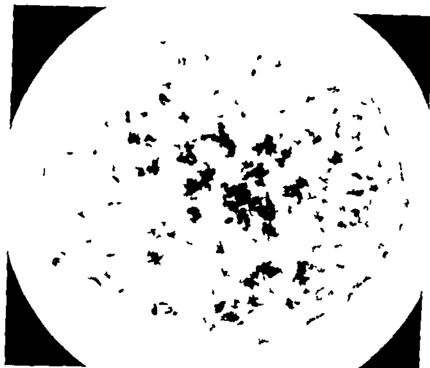


FIG 4 —Red cell agglutination by poly lysine

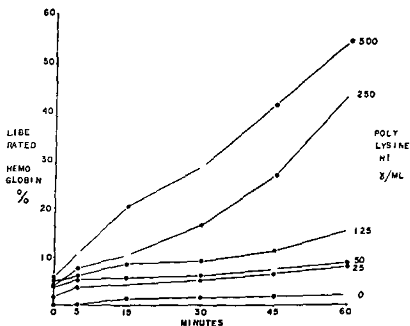


FIG 5 —Liberation of hemoglobin from red cells by poly lysine

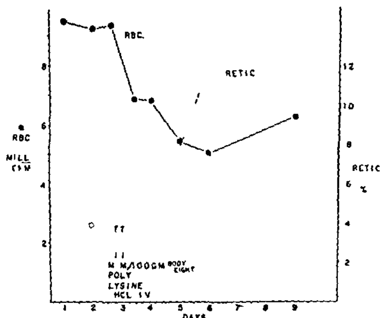


Fig 6 —Hemolysis by intravenous poly lysine in a rat

that poly lysine caused structural changes in the red cell membrane by accumulating lipid material in masses possibly by binding the polar groups of the radially oriented lipid molecules

The intravenous injection of poly DL lysine HCl in doses of 1–1 2 mg per 100 Gm bodyweight into 6 rats caused a drop in the red cell count of 12–24% in 4 rats while in 2 rats the drop was considered insignificant The fall occurred on the 3rd–4th day and was accompanied by reticulocytosis ranging up to 12% the maximum being observed on the 2nd–6th day after injection Two injections of 1 0 mg given within one half hour to one rat caused a drop in red cells of 45% and a reticulocytosis of 13% (fig 6)

The peripheral smears did not show abnormality of the red cell The bone marrow findings obtained in some of these rats usually after the red cell count had already begun to rise again were variable However in two rats marked erythroid hyperplasia was observed

Summary

Poly DL lysine HCl inhibits thrombin formation and agglutinates and hemolyzes red blood cells in vitro

Nonlethal intravenous doses of poly lysine disturb thrombin formation in shed blood and cause hemolysis in vivo

Rats given lethal intravenous doses of poly lysine die with pulmonary edema and neurologic disturbances but do not show red cell agglutination or hemorrhage

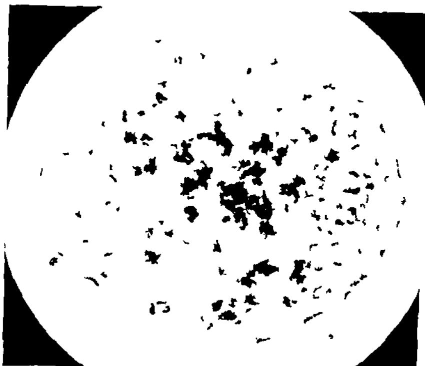


FIG 4—Red cell agglutination by poly lysine

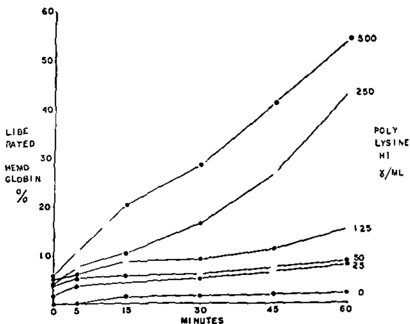


FIG 5—Liberation of hemoglobin from red cells by poly lysine

trombocítico así que nos parece justificado clasificar la *enfermedad como una mesenquimopatía hemorrágica constitucional plasmopática y trombopática a la vez*.

Como tal en la actualidad la hemofilia se halla colocada entre las formas mixtas de trombopatías o de plasmopatías hemorrágicas y precisamente en la categoría de las *plasmotrombopatías*. Es probable que el doble defecto de la hemofilia esta por una parte en la escasa producción o liberación de tromboquinasa de las plaquetas y por otra parte en la insuficiencia de una substancia plasmática necesaria para la formación de la trombina.

Las *estrechas analogías patogénicas entre hemofilia y trombopatías* no interesan es obvio a todas las formas de estas ultimas enfermedades. El campo de las trombopatías constitucionales aunque constituya ahora una realidad clínica bastante solida todavía precisa de ulteriores estudios para ser mejor aclarado. La variedad de los fenómenos acerca del padecimiento hemostático ha llevado a los primeros investigadores sobre un camino demasiado analítico. Sin embargo debemos reconocer que todavía no es posible incluir a la mesenquimopatía hemorrágica trombopatica constitucional en un unico tipo. La analogia patogénica entre hemofilia y trombopatías constitucionales es por lo tanto evidente solo en algunas formas pertenecientes a este nuevo grupo de enfermedades hemorrágicas.

VII communication 2

Recent Italian Contributions to the Study of Hemophilia

ANGELO BASERGA*

I will refer here to the most notable and recent Italian contributions to the study of hemophilia.

Among the many clinical observations it is interesting to note the coincidence of hemophilia with various morbid conditions relatively frequent in some areas of our country, such as the association studied by Masciotta (Pediatria 4? 1 1956) of hemophilia and favism in which to the hemoglobinuria proper of this disease a strong hematuria with ecchymosis was added, or that described by Tropeano (Progresso Medico 7 279 1951) of a family in which the association of hemophilia and thalassemia minor was found. Casarini (Boll Soc It Biol *13* II 1948) described the association of hemophilia with brachydactylia and Patrignani (Policlinico Sez Med *5?* 1945) that of hemophilia with Hand Schuller Christian disease.

From the viewpoint of the pathogenetic investigation of hemophilia Grifone and Vanacore have confirmed the observations of Castex and Pavlovsky of 1941 that the recalcification time of hemophilic plasmas diminishes as the time lapse between the extraction of the sample and the running of the test increases.

Crisalli and Gotelessa (Haemat *3?* 1 19?) reported in 1950 a case of pseudohemophilia due to the presence of a circulating anti coagulant in a child 10 years old. This was a heparin like substance and was neutralized with toluidine blue.

Tropeano in 1951 (Prog Med 7 15 1951) described another case of pseudo hemophilia due to an anti coagulant of the type of an antithromboplastinogen.

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Synthetic and natural acidic polymers may protect the poly lysine injected rats from death and from coagulation disturbance

Poly lysine neutralizes the clot retarding effect of heparin in vivo

Efectos Hematicos de la Poli DL lisina in Vitro e in Vivo

La Poli DL lisina HCl inhibe la formación de trombina y aglutina y hemoliza los globulos rojos in vitro

Dosis endovenosas no letales alteran la formacion de la trombina y causan hemolisis in vivo

Las ratas que reciben dosis endovenosas letales mueren con edema pulmonar y trastornos nerviosos pero no muestran aglutinacion de los hematíes ni hemorragias

Polímeros ácidos sintéticos y naturales pueden proteger de la muerte y de los trastornos de la coagulacion a las ratas que recibieron poli lisina

La poli lisina neutraliza el coágulo retardando el efecto de la heparina in vivo

VII communication I

Hemophilia and Thrombopathies

NEVIO QUATTRIN*

Classic hemophilia and various other thrombopathies have many points in common mainly when one considers the type and location of the hemorrhagic disorders and the alterations in hemocoagulation and hemostasis

Modern investigations on the humoral coagulation process while in a way confirming the theory of Morawitz emphasize on the other hand the platelet as a chief factor in hemo ta sis Classic hemophilia does not escape this rule since the disorder of the plasmatic factor does not seem sufficient to explain its pathogenesis We mu t also admit an alteration of the platelet factor so it seems justified to clas ify the diseas as a constitutional hemor rhagic mesenchymopathy both plasmopathic and thrombocytopathic

Hemophilia is nowadays classified as a mixed form of hemorrhagic thrombopathy or plasmopathy and placed in the category of the plasmo thrombopathies This dual component in hemophilia could be due to an inhibited production or liberation of thrombokinase from the platelets and in insufficiency of a plasma substance necessary for the production of thrombin The close pathogenetic analogies between hemophilia and other thrombopathies obviously do not hold for all types of these last disease Though the con titutional thrombopathies are now a fairly solid clinical entity more studies are needed to clarify the picture

Hemofilias y Trombopatias

La hemofilia clásica y varias trombopatias presentan muchos puntos de contacto principalmente por lo que se refiere al tipo y lugar de las manifestaciones hemorragicas y al padecimiento de la hemocoagulacion hemostasis

Las investigaciones modernas sobre el proceso de la coagulacion humoral mientras que por una parte confirman en sus grandes lineas la exactitud de la teoría de Morawitz Fuld y Spiro ponen por otra parte a la plaqueta como centro de la hemostasis La hemofilia misma no escapa a e ta regla por lo que el solo desorden del factor plasmatico no es suficiente para explicar su patogénesis Cabe en vez admitir también un padecimiento del factor

* Naples Italy

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that the injection of two cc of fresh plasma every twelve hours gives a satisfactory therapeutic response and have called this quantity anti hemophilic unit

Gualandi Barengo and Mari (Prog Med 7 609 1951) have used thromboplastin (Geigy s brand) in subcutaneous injections and have obtained a *decrease in clotting time*

Breda Bernardi and Sala (Giorn Clin Med 32 7 1951) have used the venom of *Bothrops Jararaca* intravenously (1 to 2 cc) and have found that this therapy can replace the use of small plasma or blood transfusions

The school of Sotgiu and Lenzi has *observed clinical improvement and reduction of the* clotting time of a hemophiliac by administering 50 to 100 micrograms of vitamin B_1 during several days

In clinical improvements some importance must be attached to the protective action of Vitamin E on the capillaries *an important factor in hemophilia stressed with authority* by Pavlovsky for some time

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We also consider the residual prothrombin test very important

The modification introduced by Seegers for the preparation of anti hemophilic globulin or thromboplastinogen or platelet co factor according to his terminology has contributed to clarify the problem of this disease

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Recientes Contribuciones Italianas al Estudio de la Hemofilia

Me referire aquí a las más notables y recientes contribuciones italianas *al estudio de la* hemofilia

Entre las muchas observaciones clinicas singulares son las descripciones de coincidencia de hemofilia con estados morbosos relativamente frecuentes en algunas zonas de nuestro país como la asociación e tudiada por Masciotta (Pediatria 46 1 1936) de hemofilia y favismo donde a la hemoglobinuria propia de esta enfermedad se asocio una fuerte hematuria con aparición de manchas equimoticas en el cuerpo o aquella descripta por Tropeano (Progresso Medico 7 799 1951) de una familia en la cual se encontró la asociación de hemofilia y thalasemia menor Casarini (Boll Soc It Biol 13 II—1948) describio la asociacion de hemofilia con braquidactilia y Patrignani (Policlinico Sez Med 52 1945) de hemofilia con enfermedad de Hans Schuller Christian

Desde el punto de vista de la investigacion patogenética de la hemofilia Crifone y Vanacore han reproducido las observaciones de Castex y Pavlovsky de 1941 de que el tiempo de recalcificacion del plasma de hemofílicos disminuye con el aumento del intervalo de tiempo transcurrido entre la obtención de la muestra y su determinación

Crisalli y Cotelessa (Haemat 36 1 19 ?) describieron en 1950 un caso de pseudo-hemofilia debido a la presencia de un anti coagulante circulante en un niño de 10 años del tipo de la heparina, neutralizado con Azul de Toluidina

Tropeano en 1951 (Prog Med 7 15 1951) describió otro caso de pseudo hemofilia debido a un anti coagulante de tipo antitromboplastinógeno

Ciannico observó una alteración del test de la consumición de la protrombina en la hemofilia con aumento de la protrombina residual en el suero en el 60% de los casos estudiados

Conte Marotta Tropeano y Postiglione (Progresso Med 6 13 19 0) han preparado y usado en Italia la globulina anti hemofílica y han también demostrado su importancia diagnóstica cuando acorta el tiempo de coagulación en casos dudosos de hemofilia

Bianchi y Cataldi han estudiado el efecto terapéutico del plasma fresco y han encontrado que la inyección de 2 cm³ de plasma fresco cada 12 horas obtiene una respuesta terapéutica satisfactoria y han llamado a esta cantidad unidad anti hemofílica

Cualandi Barengo y Mari (Prog Med 7 609 1951) han usado la tromboplastina Ceigy inyectada subcutáneamente obteniendo disminuciones del tiempo de coagulación

Breda Bernardi y Sala (Giorn Clin Med 3 7 1951) han usado el veneno de la víbora Bothrops Jararaca por vía endovenosa (1 a 2 cm³) y han encontrado que esta terapéutica puede reemplazar el uso de pequeñas transfusiones de sangre o plasma

La escuela Sotgiu y Lenzi ha observado mejoramiento clínico y reducción del tiempo de coagulación en un hemofílico mediante el suministro de 50 a 100 microgramos de Vit B12 durante varios días

En el mejoramiento clínico debe tener importancia también la acción protectora de la Vit P sobre los capilares factor importante en la hemofilia según lo ha venido sosteniendo con autoridad Pavlovsky

Hemos ya mencionado (Baserga y De Nicola Lancet 1951/II 1039) la observación de que con transfusiones aun en el orden de 2 a 3 cc /kg en hemofílicos se acorta el tiempo de coagulación a cifras normales pero el serum prothrombin time permanece elevado Creemos que en los casos donde debe hacerse cirugía conviene normalizar ambos tiempos

Considerando ahora el problema general de la hemofilia desde el punto de vista patogenético las dos principales contribuciones en los últimos años son la identificación de la globulina anti hemofílica en 1936 y la observación de Quick en 1947 Según este último autor el factor plaquetario o tromboplastinogenasa reacciona con el factor plasmático o tromboplastinógeno para formar tromboplastina Si el factor plasmático es deficiente como sucede en la hemofilia no se forma suficiente tromboplastina y todo el proceso de la coagulación está afectado Esto explica por qué la hemofilia ha sido considerada por largo tiempo como una enfermedad por déficit de tromboplastina Como se forma poca trombina ésta no es suficiente para desintegrar las plaquetas que parecen además ser más resistentes

Damos suma importancia también al test de la protrombina residual

La modificación aportada por Seegers para la preparación de la globulina anti hemofílica o tromboplastinógeno o co factor plaquetario según su nomenclatura ha contribuído en forma notable a la clarificación del problema de esta enfermedad

En conclusión los puntos sobre los cuales debe polarizarse la atención de los investigadores en el campo de la hemofilia deben ser los siguientes desarrollo de la teoría de Quick del año 1947 con las mejoras propuestas por Seegers en 1952 o técnicas análogas que se están investigando en estos momentos profundización del concepto actual sobre la eventual acción aceleradora de la globulina anti hemofílica ulterior desarrollo y perfeccionamiento de los tests diagnósticos y finalmente reconociendo nuestra actual impotencia en el tratamiento de esta enfermedad la investigación de nuevas formas terapeuticas aparte de las actuales transfusiones de sangre

that the injection of two cc of fresh plasma every twelve hours gives a satisfactory therapeutic response and have called this quantity anti hemophilic unit

Gualandi Barengo and Mari (Prog Med 7 609 1951) have used thromboplastin (Geigy s brand) in subcutaneous injections and have obtained a decrease in clotting time

Breda Bernardi and Sala (Giorn Clin Med 32 7 1951) have used the venom of *Bothrops Jararaca* intravenously (1 to 2 cc) and have found that this therapy can replace the use of small plasma or blood transfusions

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durante el embarazo sea una respuesta a la muerte fetal intrauterina o algún otro fenómeno que represente una intolerancia de la madre a los productos de la concepción.

La esplenectomía se recomienda como tratamiento de elección para la madre en los casos de púrpura trombocitopénica idiopática como complicación de un embarazo cualquiera sea el período de gestación.

En el recién nacido puede ser también aconsejable a veces la esplenectomía, pero debe ser precedida por un período de tratamiento no quirúrgico, pues el restablecimiento espontáneo suele ser la regla en el curso de la enfermedad.

La ACTH ha sido útil como ayuda terapéutica, pero no provocó remisión de la enfermedad en ninguno de los dos casos de esta serie en que se usó.

VII-communication 4

Regulación de Hemostasia y Prevención de Trombogénesis

H. W. KEMPSKI*

Esta comunicación se refiere a observaciones y experiencias con la flora intestinal patológica y fisiológica en su relación a la producción de Vitamina K.

Trata sobre la prevención y el combate de la disposición para trombosis, tromboflebitis, etc., mediante regulación de la flora intestinal productora de Vitamina K. Considera la alteración de los microorganismos fisiológicos del intestino grueso por quimio y antibióticoterapia. Describe la posibilidad de regular la regeneración de *coli-bacilos* fisiológicos productores de Vitamina K. Cepas fisiológicas de *Escherichia coli* pueden ser agregadas e introducidas mediante el método descripto por el autor.

Regulation of Hemostasis and Prevention of Thrombogenesis

This communication refers to observations and experiments on intestinal flora, both physiological and pathological, in relation to its production of vitamin K.

The subject matter is prevention and fight against a disposition towards thrombosis, thrombophlebitis, etc., by regulating the intestinal flora which produce vitamin K. Considerations are made on the alteration of physiological microorganisms of the colon by chemical and antibiotic therapy. It describes the possibility of regulating the regeneration of physiological *coli-bacillus* producers of vitamin K. Moreover, physiological stocks of *Escherichia coli* may be added and introduced by the method described by the author.

* Asociación Argentina para el Estudio de las Enfermedades Transmisibles, Misiones, Argentina.

VII communication 3

Splenectomy in Pregnancy for Thrombocytopenic Purpura

ROBERT S SPARKMAN, JORGE LAJOUS and
J M HILL*

Splenectomy during pregnancy for thrombocytopenic purpura has been described in twelve reports covering thirteen cases (eleven for primary purpura and two for secondary purpura) Three additional cases are reported at this time bringing the total to sixteen cases A report of splenectomy for purpura in the newborn is also included This is thought to be the first report of splenectomy in the newborn following previous splenectomy of the mother for purpura

The over all operative and obstetric mortality for the sixteen mothers is zero The fetal mortality is 25 per cent and is derived entirely from the first four cases to be reported

The survival rates are distinctly better in splenectomized patients than in those treated by non surgical measures While this is more notably true of maternal survival an increased salvage of the infant is also apparent

Splenectomy in the purpuric mother does not prevent the appearance of purpura in the infants of subsequent pregnancies However since infant mortality rates from this cause are low in subsequent pregnancies it may be inferred that some degree of quantitative protection is afforded to subsequent children

In selected cases it is possible that the initial development of purpura during pregnancy is a response to fetal death in utero or to some other phenomenon representing an intolerance of the mother to the products of conception

Splenectomy is recommended as the treatment of choice for the mother in instances of thrombocytopenic purpura complicating pregnancy regardless of the period of gestation Splenectomy of the newborn may also be advisable at times but should be preceded by a period of non operative treatment since spontaneous recovery is the usual course of the disease ACTH has been useful as a therapeutic aid but has not induced remission of the disease in either of two cases in this series

La Esplenectomia por Purpura Trombocitopenica Idiopatica en el Embarazo

La esplenectomía por purpura trombocitopénica durante el embarazo ha sido descripta en 12 trabajos que recogen 13 casos (11 por purpura primaria y 2 por purpura secundaria) Tres nuevos casos se refieren ahora con lo que el numero asciende a 16 casos Se incluye también un caso de esplenectomía por purpura trombocitopénica en un recién nacido cuya madre había sido esplenectomizada por la misma causa Esta situacion sería descripta por primera vez en la literatura en el presente trabajo La mortalidad global para las 16 madres *es cero La mortalidad fetal llega al 25% correspondiendo* en su totalidad a los primeros 4 casos referidos

Las supervivencias son evidentemente superiores en los casos esplenectomizados que en los tratados por métodos no quirurgicos especialmente por lo que se refiere a las madres aunque ésto cabe también para los ninos

La esplenectomía en las madres con purpura trombocitopénica no impide la aparición de purpura en los ninos de subsiguientes embarazos Sin embargo somo la mortalidad infantil se reduce en los proximos embarazos puede deducirse que se consigue cierto grado de proteccion cuantitativa para los futuros ninos

En determinados casos es posible que el desarrollo inicial de purpura trombocitopénica

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Department of Surgery Baylor Hospital and the J K and Susie L Wadley Research Institute and Blood Center Dallas Texas U S A

durante el embarazo sea una respuesta a la muerte fetal intrauterina o algún otro fenómeno que represente una intolerancia de la madre a los productos de la concepción

La esplenectomía se recomienda como tratamiento de elección para la madre en los casos de púrpura trombocitopénica idiopática como complicación de un embarazo cualquiera sea el período de gestación

En el recién nacido puede ser también aconsejable a veces la esplenectomía pero debe ser precedida por un período de tratamiento no quirurgico pues el restablecimiento espontáneo suele ser la regla en el curso de la enfermedad

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VII communication I

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* Asociacion Argentina para el Estudio de las Enfermedades Transmisibles Misiones Argentina

VII communication 5

Influencia del Veneno de Serpiente sobre el Tiempo de Sangría en las Enfermedades Hemorrágicas

G ROSENFELD y D DE CILLE*

El veneno de Bothrops jararaca diluído aproximadamente al 1/1000 e inyectado por vía intramuscular o endovenosa tiene una accion evidente sobre el tiempo de sangría acortándolo en pacientes con enfermedades hemorrágicas con trombocitopenia y en un hemofílico con tiempo de sangría prolongado

En ciertos casos en los que el tiempo de sangría se encuentra marcadamente prolongado la dosis usual de 1 m l puede provocar una fase inicial o tardía negativa Este riesgo puede ser evitado inyectando dosis muy pequeñas de 0 1 0 05 o 0 025 m l

Estas pequenas dosis son ya suficientes provocando solamente el acortamiento del tiempo de sangría

La fase negativa no se produce con la dosis normal en los casos en que dicho tiempo no se halla muy prolongado

INFLUENCE OF SNAKE VENOM ON THE BLEEDING TIME IN HEMORRHAGIC DISEASE

Bothrops jararaca venom diluted approximatedly to 1/1000 and injected intramuscularly or intravenously has a clear action on the bleeding time shortening it in patients with hemorrhagic diseases with thrombocytopenia and in a hemophilic with prolonged bleeding time

In certain cases in which the bleeding time is markedly prolonged the usual dose of 1 ml may cause an initial or late negative phase This risk can be eliminated when very small doses of 0 1 0 05 or 0 025 ml are injected Even these small doses are efficient inducing only a shortening of the bleeding time The negative phase does not occur with normal doses in cases in which no prolonged bleeding time is observed

Laboratorio de Hematología Instituto Butantan São Paulo Brazil

VII communication 6

Direct Platelet Counting

R FEISSLY† and H LUDIN‡

Most authors still regard the counting of blood platelets as one of the most unreliable procedures of clinical hematology (G J Scheff and P H Ralph)

As a matter of fact only direct methods may claim sufficient accuracy provided however that certain points be taken into account namely

1 The use of the phase microscope in order to secure the visibility of platelets

2 The use of a blood diluent capable of hemolyzing red blood cells while preserving the platelets such a fluid should also prevent clumping of the platelets and adhesion of these formed elements to glass surfaces

Since cocaine salt present these features we use a diluent a solution of 1/5 isotonic NaCl (buffered with sodium phosphates pH 4) with the addition of 3% cocaine chlorhydrate

† University of Lausanne and ‡ Medical Department University of Basle

Blood is diluted 1 20 in a leukocyte pipet it is homogenized and then allowed to stand in the pipet for 15-20 minutes to allow for sufficient hemolysis Then it is homogenized again and a counting chamber of the Thoma type is charged (if possible the latter should be 0 05 mm deep) After waiting for 10-15 minutes—in order to obtain adequate sedimentation—the counting is performed under phase contrast (Objective PH 40—X420 or more)

The platelets appear as distinct regular edged dark disks homogeneously distributed without any trace of clumping

Recuento Directo de las Plaquetas

Casi to los los autores aun consideran que el recuento de plaquetas es uno de los procedimientos menos seguros de la clínica hematologica (G J Scheff y I H Ralph)

Se considera que solo los métodos directos pueden ser considerados seguros siempre que ciertos detalles sean tomados en consideración

1 El uso del microscopio de fase para asegurar la visibilidad de las plaquetas

2 El uso de un diluyente de la sangre capaz de hemolizar los glóbulos rojos preservando las plaquetas este líquido debe también prevenir el aglutinamiento de las plaquetas y la adhesion de las mismas a las superficies del vidrio

Ya que las sales de cocaína presentan estas características usamos como diluyente una solución de 1/5 de NaCl isotonico (mezclado con fosfato de sodio a un pH 7 4) agregándole 3% de cocaína clorhidratada

La sangre es diluída 1 20 en una pipeta para contaje de blancos es homogeneizada y se deja en la pipeta de 15 a 20 minutos para conseguir la suficiente hemolisis Luego se homogeiniza nuevamente y se carga una cámara del tipo Thoma (si es posible de 0.05 mm de profundidad) Después de esperar de 10 a 15 minutos para obtener la adecuada sedimentacion el recuento se hace bajo contraste de fase (Objetivo PH 40—X420 o más) Las plaquetas aparecen con bordes regulares como discos oscuros homogéneamente distribuídos y sin aglutinamientos

VII-communication 7

Acquired Temporary Afibrinogenemia

G KENT*

A case of a boy aged three years who developed a serious hemorrhagic diathesis three days after the onset of a sore throat is reported Massive hemorrhages with necrosis were present in the face and all extremities There was also considerable hematuria Examination of the blood revealed complete absence of fibrinogen Transfusions were of no avail and the hemorrhages were only stopped a few days later after the administration of fibrinogen which had been flown in from Boston By that time it was necessary to amputate both legs which had become completely gangrenous After that the boy made a complete recovery and has now been well for over a year showing also a normal fibrinogen concentration The fibrinogen was supplied by Dr Louis K Diamond Boston

The blood was thoroughly tested for anticoagulants and lack of other clotting factors The diagnosis of afibrinogenemia is considered absolutely established A fibrinolysin could not be demonstrated Cryoglobulins and self precipitating proteins were encountered Full hematological chemical and histological details are available together with a discussion on the etiology

Department of Pathology Moose Jaw General Hospital Moose Jaw Saskatchewan Sask Canada

In summary

1 The association of purpura fulminans with complete absence of fibrinogen is described

2 A number of noteworthy findings are recorded decreased prothrombin concentration increased thrombin inhibitory activity of plasma highly elevated anti streptolysin-O titer presence of cryoglobulins and self precipitating proteins increase in bone marrow of eosinophils and plasma cells and histological findings of necrotizing angiitis and massive thromboses

3 The pathogenesis of the afibrinogenemia is discussed

AFIBRINOGENEMIA ADQUIRIDA TEMPORARIA

El caso de un niño de 3 anos de edad que adquirió una diátesis hemorrágica seria 3 dias después de la iniciación de un dolor de garganta Presentaba grandes hemorragias con necrosis en la cara y extremidades Hematuria considerable El examen de sangre revelo ausencia completa de fibrinogeno Las transfusiones no dieron ningun resultado y las hemorragias solo fueron detenidas algunos días más tarde después de la administracion de fibrinogeno el cual había sido enviado por vía aérea desde Boston Por ese tiempo fué necesario amputarle ambas piernas las que estaban completamente gangrenadas Después de eso el niño mejoro completamente y se conserva bien después de más de un ano mostrando también una concentracion de fibrinogeno normal Se buscó cuidadosamente en la sangre la presencia de anticoagulantes o la ausencia de otros factores coagulantes Se establecio el diagnóstico absoluto de afibrinogenemia No se pudo demostrar la presencia de una fibrinolisina Fueron halladas crioglobulinas y proteinas auto precipitables Se darán todos los detalles hematologicos quimicos e histologicos así como una discusion sobre la etiología

En sumario

1 Se describe un caso de asociacion de purpura fulminans con ausencia completa de fibrinogeno

2 Se recalcan una serie de hechos dignos de mencion disminucion de la concentración de protrombina aumento de la actividad protrombino inhibidora del plasma título muy elevado de antiestreptolisina O presencia de crioglobulinas y precipitinas autoprecipitables aumento en la medula osea de eosinofilos y células plasmáticas y angiítis necrotizante con trombosis pasiva

3 Se discute la patogenia de la afibrinogenemia

VII communication 8

Physio Patho Morphology of So called Capillary Bleeding

S AMANO*

The injection of leukotoxin (Menkin) or leukomigrative (Yasuhira) subcutaneously into experimental animals results in a marked migration of leukocytes out of the venules This can be observed 15 minutes after injection by our extension method of study on connective tissue The observation of the omentum major with the extension method is suitable (Kawano 1950) for the obtainment of data relative to the changes that occur in these small vessels In this case there is an exclusive migration of leukocytes from the venulary portion while neither the capillaries nor arterioles participate in this phenomenon (Newly formed capillaries of granulation tissues are similar to venules with respect to their vascular permeability Amano Kawano 1950)

If precise observations are made on the boundary between capillaries and venules there is found a clear difference between these two parts The wall of the capillary is formed by a

* Pathological Institute Kyoto University Japan

thin smooth membrane of endothelium while that of the venule is a thick wooly mantle of adventitia There is no gradual transition between these two portions corresponding to the model illustrations in the physiology texts Due to the narrow lumen of the capillary and the tight fitting of its endothelial lining leukocytes cannot pass through its walls On the other hand although the walls of venules are thick their endothelium muscle cells and adventitia cells are loosely combined allowing for the passage of leukocytes as well as coarse particles such as carbon to pass through the wall without difficulty

If vascularly deranged mice or rabbits are injected intravenously with carbon particles the particles pass through the adventitial space just beneath the adventitial cells and are engulfed by histiocytes that are distributed in the connective tissues along the venules (Hirata and Nakajima 1940)

The following permeability test of dye stuff is recommended Cut the abdominal skin of a mouse just after intravenous (tail veins) injection of trypan blue The skin is extended to the lateral sides with caution so as not to injure the blood vessels Observation reveals the permeation of the trypan blue principally along the venules (Amano 1950) The same venular permeation may be observed with the dye uranin even though this dye tends to combine with the albumin of the circulating blood This permeation of the dye albumin complex indicates possibly that albumin is capable of passing through the venule walls in a physiological condition Most of the dye outside the venules is passed on to the lymph vessels which parallel these venules

Another phenomenon of the venules that is worthy of note is that the so called capillary bleeding or parenchymatous bleeding takes place only at a portion of the venules We first noticed this fact during some experiments on intra vital observations on the circulation of the mesenterium When the mesenterium was observed for more than two hours we saw ecchymosis of the tissue Such bleeding was confined to the venules and did not appear in the capillaries or arterioles On the basis of these findings we tested various experimentally produced bleedings which have been classified as capillary bleeding

At first observations were made on cutaneous bleeding produced by the negative pressure applied in the clinical test of capillary resistance This was observed by cutting the skin of the mouse immediately after the production of cutaneous ecchymosis The inside tissues of the skin were examined under the microscope The process of bleeding was followed precisely and was found to be confined to the venules neither capillaries nor arterioles took part in the phenomenon If the mouse was injected intravenously with india ink the experiment showed the black staining of the venule walls and the thrombus inside the bleeding vessels

The second observations were made using anti platelet sera to provoke purpura in guinea pigs With such sera we could elicit marked ecchymosis and bleeding in the skin omentum and various organs We were able to obtain and observe fine extension specimens of omentum with ecchymosis Without exception the bleedings were confined to the venules

The third set of observations were obtained from guinea pigs that had been infected with Leptospira icterohemorrhagica The bleedings that occur in these injected animals coincided with the findings in the experimentally produced thrombopenic purpura

In contradistinction to the above observations we found that the hemorrhage producing extract fraction (Hirata 1950) of tubercle bacilli elicited a decidedly different type of experimental bleeding In this case dilation and meandering of paralyzed venules was very remarkable and in some parts of these venules diapedesis bleeding appeared

The experimental findings presented in this paper namely the capillary parenchymatous bleeding is nothing but a bleeding from the venules This is very important because hitherto neither pathologist nor physiologist have described it with accuracy

Fisiopatología de la Llamada Hemorragia Capilar

Los experimentos realizados en animales muestran que la llamada hemorragia parenquimatosa capilar no es otra cosa que una hemorragia de vénulas hecho importante que hasta ahora no había sido observado con exactitud por fisiólogos o patólogos

PART VIII

Miscellaneous

Varios

Classification of Anemias

RODOLFO ARMAS CRUZ*

THERE ARE a great many classifications of anemias[1] This indicates that no single one has achieved majority use in daily clinical practice or in the teaching of this chapter in Internal Medicine One of the oldest classifications divides anemias as primaries or secondaries primaries were those whose cause was unknown and who depended fundamentally from a disorder of the hemopoietic organs and secondaries were those whose cause was known and were attributed to pathology of any other organ They were also classified as plastics and aplastics depending on the existence of blood or medular regeneration Subsequently there appeared the morphologic classifications of anemias some were based on the size of the erythrocytes and others on the quantity of hemoglobin Those based on size were divided into macrocytic microcytic and normocytic and those based on the quantity of hemoglobin into hyperchromic hypochromic and normochromic Usually macrocytics are hyperchromic microcytic are hypochromic and normochromic Usually macrocytics are hyperchromic microcytic are hypochromic and normocytic are normochromic this outline however may not be so simple e g the microcytic hyperchromic anemias (some of the hemolytic anemias)

Finally in the last few years the classification most used has been that of Minot and Castle[2] which divides anemias as (a) by *loss or destruction* of blood (hemorrhage or hemolysis) (b) a decrease in the production of blood that could be due to (1) lack of nutrition of the hemopoietic organs (2) a toxic or infective inhibition (3) damage by physical agents (4) a mechanical interference by the outgrowth of a strange tissue in the interior of the hemopoietic organs or (5) an idiopathic disturbance of the same

A similar etiologic classification is presented by Wintrobe[3] He divides anemias into 4 large groups (1) by loss of blood (acute or chronic hemorrhages) (2) by excessive destruction of blood (hemolytic anemias of intra and extracorpuscular origin) (3) by decreased blood production due to (a) a deficiency of substances concerned in erythropoiesis (iron proteins and various elements of vitamin B) or (b) some fault in the construction of red corpuscles (infection various chronic diseases physical injury intoxication endocrines hypersplenism myelophthisis) and (4) congenital dystrophies concerned with hemolytic intracorpuscular anemias (congenital hemolytic jaundice sickle cell anemia and Mediterranean anemia)

All these classifications are good but they all have defects I do not pretend that my classification is the best one, I only think that it facilitates learning of the basic outline To make this classification I have started from Dameshek s idea of considering the bone marrow as a factory that produces the red blood

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marrow However the phenomenon of the anemia is determined by post-medullary reason that is by the destruction of these cells This is proved in the microspherocytic anemia the phenomenon is cured simply by splenectomy

It could also be said that the hypochromic anemias presented as premedullary are frequently postmedullary as in most cases they are due to chronic hemorrhage This is true but it is further true that the essential mechanism is also in the lack of iron in chronic hemorrhage when sufficient iron is administered anemia is not produced Finally anemias of some illnesses (cirrhosis leukemia Hodgkin pregnancy etc) are located in different groups but this is because their mechanism may be multiple

We only want to outline these facts in a practical form and we are the first to admit that the outline is vulnerable to criticism

TABLE 1—*Classification of Anemias*

Premedullary

(1) By lack of iron (microcytic and hypochromic)
(2) By lack of a *vitaminic factor*
   (a) a maturative factor of Vitamin B (macrocytic and hyperchromic)
      Vitamin B_{12} (pernicious anemia)
      Folic acid (sprue tropical anemia of pregnancy bowel intestinal dysfunction)
      Nicotinic acid (pellagra)
   (b) lack of Vitamin C
(3) By lack of a hormonal factor hypothyroidism Addison s disease and others

Medullary

(Lanext penia refractary anemias usually normocytic and normochromic)
(1) Hypoplastic and aplastic anemias
   (a) primitives or idiopathic
   (b) secondary
      1 physical damage
         a external irradiation (x rays radium)
         b internal irradiation (radioactive substances P I Na)
      2 chemical damage
         a toxic (nitrogen mustard TEM)
         b intolerance or allergy (Benzol and derivatives Neo alvarsan Chloromycetin sulphas etc)
(2) Bone marrow occupation (myelophthisis)
   (a) by extrinsic tissue carcinomatous metastasis xanthomatosis and similar marble bone disease
   (b) by intrinsic tissues Hodgkins lymphosarcoma reticuloendotheliosis leukemias multiple myeloma myelofibrosis or myelosclerosis

Postmedullary

(with hyperactive marrow)
(1) by hemorrhage
   (a) acute
   (b) chronic
(2) *by hemolysis*
   (a) intrinsic cause (intracorpuscular) congenital
      1 congenital hemolytic jaundice (microspherocytic) or Chauffard Minckowsky s disease

cells It may be outlined as follows (1) that the factory requires raw material (2) that it synthesizes these materials, and then (3) that it gives the elaborated material for use and destruction

With this concept anemias may be divided into 4 fundamental groups (1) premedullary anemias, in which absence of formation is due to a lack or deficiency of the raw material (2) medullary anemias where the incapacity is in the bone marrow itself (3) postmedullary anemias in which the trouble is due to a loss or destruction of the blood (hemorrhage hemolysis) (4) anemias of uncertain causation in which the trouble is not clearly situated, and therefore, they are not premedullary medullary or postmedullary

This classification does not prejudge the size or quantity of hemoglobin of the hematies nor as to whether there is or is not regeneration of the blood It could be said that it is anatomic and physiopathological at the same time and it has the advantage of being very simple to learn, that is, it is nemotechnic and hence didactic Experience has taught us that this type of anatomic functional classification is useful and has been well received in clinical practice This has happened with Fishberg's classification of uremias as pre renal renal and post renal, the same thing has happened with jaundice classified by Ducci as pre hepatic, hepatic and post hepatic [3]

We have divided anemias then as (1) premedullary (2) medullary (3) post medullary (4) uncertain causation (secondary anemias and anemias by hypersplenia) Table 1 enlarges upon this arrangement

Premedullary anemias are represented by those in which there is a lack of a necessary substance in the construction or maturation of the red blood cells These are the principal ones (a) iron deficiency (b) deficiency of a vitaminic factor (c) failure of some hormonal factor

Medullary anemias are represented by the clinical features of decrease or atrophy of the medular tissue (primary or secondary) or by substitution of the medular tissue by a foreign tissue (myelophthisic anemias) it can be from the exterior (metastasis lymphosarcoma etc) or by the pathologic hyperplasia of one of the same hemopoietic elements of the bone marrow (osteo clerosis and myelosclerosis)

Postmedullary anemias are represented by anemias due to hemorrhage and to hemolysis

Two groups of imprecisely understood anemias could not be included in any of the foregoing three classes (1) the so called secondary anemias whose mechanism of production is not clear and is probably multiple—that is there would be a failure of the maturative elements of the red blood cells (premedullary) of the bone marrow itself (medullary) and besides a peripheric trouble (post medullary) and (2) anemias from hypersplenism which are probably medullary but it is possible that in their production other factors may interfere

We have to admit that this classification is open to discussion For instance in the postmedullary anemias of hemolytic origin of intrinsic cause it could be said that they are medullary inasmuch as the hemolysis is produced because the red blood cells have a deficient contexture that is elaborated by the bone

Haciendo una comparación con la clasificación de las ictericias en prehepáticas hepáticas y posthepáticas se propone dividir las anemias en 4 grupos (1) Anemias premedulares (2) Anemias medulares (3) Anemias postmedulares (4) Anemias de mecanismo mal precisado (anemias secundarias)

Esta clasificación tendría la ventaja de ser muy sencilla para el aprendizaje de los estudiantes y de ser fisiopatológica y citológica al mismo tiempo no prejuzgando acerca del tamaño celular acerca de la concentración de hemoglobina del glóbulo rojo ni del principio madurativo que estaría fallando

Las anemias premedulares estarían representadas las en que falta alguna substancia necesaria para la construcción o maduración del glóbulo rojo siendo tres las principales (1) por carencia de hierro (2) por carencia de algún factor vitamínico y (3) por falta de algún factor hormonal

Las anemias medulares estarían representadas por los cuadros de disminución o atrofia del tejido medular (primitiva o secundaria) o los de substitución del tejido medular por un tejido extraño (mielotísicas sea del exterior de la médula o por la hiperplasia patológica de uno de los propios elementos hematopoyéticos medulares

Las anemias postmedulares estarían representadas por las anemias por hemorragia y por las anemias por hemólisis

Quedarían fuera de la clasificación anterior dos grupos de anemias no bien catalogadas (1) las llamadas anemias secundarias cuyo mecanismo de producción no es claro y es probablemente multiple es decir que habría una falla de los elementos constructivos del glóbulo rojo de la médula misma y además un trastorno periférico y (2) las anemias por hiperesplenia las cuales es posible que sean medulares pero es posible que en su producción también intervengan otros factores

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VIII 2

Sickling of the Red Cells in Oriental Jews

F DREYFUSS, J MUNDEL and M BENYESCH*

IT IS the purpose of this communication to review our experience accumulated within the last two years concerning the appearance of the sickle cell trait in the Oriental Jewish population in Israel We furthermore wish to outline some of the implications of these findings as to the phenomenon of sickling in general and as to their possible anthropological significance

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The senior author gratefully acknowledges the splendid cooperation of Dr G Mundel and Dr M Benyesch without whose help this study would not have been possible Thanks are also due to our technician Alisa Moshe

2 sickle cell anemia
3 Mediterranean anemia or thalassemia or target cell anemia
4 Miscellaneous with ovalocytosis with macrocytosis with astrocytosis
(b) extrinsic cause (extracorpuscular) acquired
without hemoglobinuria
with hemoglobinuria
1 infectious
2 parasitic
3 toxic or chemical
4 allergic
5 physical agents
6 vegetable poisons
7 animal poisons
8 by diseases of unknown causation (Hodgkins lymphosarcoma leukemias cancers lupus erythematosus cirrhosis ovarian cysts etc)
9 by immunologic reactions of known or unknown causation
a with known antibodies
b with unknown antibodies
10 hemoglobinurias by cold nocturnal paroxysmal of marching

Anemias of Uncertain Causation

(1) The so called secondary anemias (usually normochromic normocytic and refractory)
(a) of the infectious diseases
1 local abscess suppurative pleurisy chronic osteomyelitis chronic pyelonephritis pelvis inflammation hepatic abscess regional tuberculosis etc
2 general
a acute (typhoid fever brucellosis)
b chronic (subacute bacterial endocarditis brucellosis)
(b) diseases of unknown origin but possibly due to infectious or immunologic cause (rheumatoid arthritis lupus erythematosus etc)
(c) diseases of poorly understood origin
1 tumors and similar diseases (Hodgkins leukemias etc)
2 hepatic cirrhosis
(d) toxic cause
1 endogenous (chronic uremia)
2 exogenous (sulfas etc)
(e) chronic pancreatic diseases
(f) of pregnancy
(2) Anemias by hypersplenism
(a) parasitic (kala azar chronic paludism etc)
(b) microbic (subacute bacterial endocarditis brucella etc)
(c) of deposit (xanthomatosis and similar)
(d) Hodgkins and others
(e) congestive splenomegaly

CLASIFICACION DE LAS ANEMIAS

Hasta este momento no existe un criterio uniforme para la clasificación de las anemias La division en primitivas y secundarias y en plásticas y aplásticas ya no puede seguir en uso La clasificacion segun la carga de hemoglobina de los globulos rojos que las divide en hipercromicas hipocromicas y normocromicas tambien tiene defectos La clasificación morfológica que las divide en macrocíticas microcíticas y normocíticas y la fisiopatológica que las divide en anemias con medula funcionalmente hiperactiva (por hemorragia o por hemolisis) y en anemias con médula funcionalmente hipoactivas (nutritivas tóxicas mielotísicas e idiopáticas) son las clasificaciones más en uso actualmente

Haciendo una comparacion con la clasificacion de las ictericias en prehepáticas hepáticas y posthepáticas se propone dividir las anemias en 4 grupos (1) Anemias premedulares (?) Anemias medulares (3) Anemias postmedulares (4) Anemias de mecanismo mal precisado (anemias secundarias)

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VIII 2

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Method

Our technique for the determination of the presence of sickle cells has been the classical method of Scriver and Waugh[1] throughout five minutes of stasis of the finger preceding the taking of the blood The sealed specimens were kept at 37° first but slightly lower temperatures (32–33) proved to be more suitable At least three samples were taken simultaneously from each individual Sickling, when present was usually observed after 24–36 hours but occasionally earlier

Four hundred and four Oriental Jewish children were examined comprising mostly immigrants from Yemen, but also some from Iraq Kurdistan Iran and a small number from North Africa We were able to report at the beginning of this investigation that seven children had been found to show the sickle cell trait among the first one hundred and five Yemenite children examined A report now in press[3] summarizes the results of the whole group including the various mentioned communities 45 sicklers 2 of them possibly suffering from sickle cell anemia were found to show sickle cells (11 1%) and in addition 23 of their 73 family members examined (adults and children) exhibited the same findings

In a much smaller survey of women carried out on the obstetrical ward of our hospital a young healthy Kurdish Jewish woman (K Ch) was found to exhibit intensive sickling after about 24 hours

Dr W Hirsh[4] in a recent survey of the sickling trait among a group of 100 children on the Pediatric Ward of the Tel Aviv Municipal Hospital found two children showing sickle cells both of oriental Jewish descent

Throughout the work we were struck by a certain inconstancy in our findings Although in the above figures only instances where sickling was observed on at least two occasions are included we incurred more specimens positive on only one occasion This seemed to us the more puzzling since the only Arab in whom we had been able to find sickle cells in the course of a survey of Arabs and Bedouins[5] in analogy to the findings in African sickling showed constant sickling in all specimens and on every examination He furthermore showed sickle cells when his blood was exposed to ascorbic acid exactly in the way that African sickling behaves Several attempts to produce sickling by any of the reducing agents (sodium hydrosulfide[6] sodium metabisulfite ascorbic acid B subtilis culture[8] or stool extract[9]) failed in the individuals examined Furthermore in a group of 104 Yemenites examined for the presence of the sickle cell trait by means of reduction only (sodium metabisulfite) no instance of sickling was discovered[10] The Scriver Waugh method was inapplicable under the special circumstances

Discussion

We are therefore left with a number of problems to be cleared up in the future

Is this type of sickling identical with the classic African type of the disease? In other words are the inconstancy of the findings and the lack of production of the sickle cell phenomenon by reducing agents enough to make this type of sickling an almost separate entity?

Recently inconstancy of the sickling phenomenon seems to have been occasionally noted even in Negroes [11] The idea that conditions in the plasma and its composition play a certain part in the bringing out of sickling is somewhat supported by the study of Lange and his associates[12] and although in a much cruder fashion by Isaacs [13] production of a sickle like deformity of the red cells by addition of gelatin Benyesch[14] and Lehmann[15] have both suggested that on incubation certain products may form in the plasma of these individuals which may be capable of inducing the change of shape of the erythrocyte apart from the conditions of reduced oxygen and pH decrease acting on red cells containing specific sickling hemoglobin[16] to which this change is usually ascribed We will attempt to work on this problem mainly by electrophoretic studies of hemoglobin and plasma It is possible that our findings as well as some of those of Cammopetros[17] in a large number of Greeks where he also apparently encountered the same inconstancy could be explained by a more complex interrelation of plasma and an abnormal hemoglobin both contributing to the development of sickling

The number of instances reported with non African sickling has grown steadily within the last few years Tens of cases of sickling have been observed recently in Greece and Italy or people of Mediterranean descent [18, 19] Our own findings comprise individuals from several mostly eastern Mediterranean countries We have in our first publications on this subject [9] explained our arguments—historical anthropological and others—which strongly support the thesis voiced by others [1] as well as ourselves and recently supported by the most interesting finding of Lehmann and Cutbush [3] in India that sickling can no longer be regarded as an exclusively African genetic character In a recent editorial in The British Medical Journal [4] it is suggested that various peoples as for instance in this case the Irulas in India and on the other hand African Negroes may have derived their sickling gene from a common ancestor or developed it in a fashion entirely independent from each other A study of the blood groups [10] and especially of the Rh phenotypes of the 101 Yemenites investigated who constitute the bulk of the sickling individuals so far encountered by us seems to exclude Negro ancestry and to put these people into the line of the white Mediterranean population This has been suggested also by the anthropologist C S Coon [5] on grounds of physical measurements years ago

Whether the sickling phenomenon incurred in the Mediterranean area is identical with African sickling or a slightly different entity—as for instance the end result of hemolysis seems identical in such different conditions as familial spherocytic jaundice or acquired hemolytic anemia—is still an open question In our opinion this type of sickling cannot be adequately explained by an admixture of Negro blood and it apparently belongs to a different group of peoples

Summary

The results of our sickle cell trait surveys in Oriental Jews are reviewed Sickling has been found in several Oriental Jewish communities

Certain differences in the behavior of the sickling phenomenon in these individuals taken together with the particular ethnic incidence of this type of

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The number of instances reported with non-African sickling has grown steadily within the last few years. Tens of cases of sickling have been observed recently in Greece and Italy or people of Mediterranean descent.[17, 18] Our own findings comprise individuals from several mostly eastern Mediterranean countries. We have in our first publications on this subject[6] explained our arguments—historical anthropological and others—which strongly support the thesis voiced by others[1, 2] as well as ourselves and recently supported by the most interesting finding of Lehmann and Cutbush[3] in India that sickling can no longer be regarded as an exclusively African genetic character. In a recent editorial in The British Medical Journal[4] it is suggested that various peoples as for instance in this case the Irulas in India and on the other hand African Negroes may have derived their sickling gene from a common ancestor or developed it in a fashion entirely independent from each other. A study of the blood groups[19] and especially of the Rh phenotypes of the 104 Yemenites investigated who constitute the bulk of the sickling individuals so far encountered by us seems to exclude Negro ancestry and to put these people into the line of the white Mediterranean population. This has been suggested also by the anthropologist C. S. Coon[5] on grounds of physical measurements years ago.

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Summary

The results of our sickle cell trait surveys in Oriental Jews are reviewed. Sickling has been found in several Oriental Jewish communities.

Certain differences in the behavior of the sickling phenomenon in these individuals taken together with the particular ethnic incidence of this type of

sickling suggest that sickling obtained in these cases by the moist stasis method may be of a different origin and character from the African type of the disease

Sickling en Judíos Orientales

Hemos encontrado rasgos de células falciformes (sickle cell trait) entre varios grupos de judíos orientales en quienes la mezcla con sangre negra es muy improbable por varias razones En un grupo de niños pertenecientes a estas comunidades la incidencia de estos rasgos a demostrado ser de un 11%

El fenomeno del sickling encontrado en estos individuos demostró cierta desviacion menor que el comportamiento del sickling en africanos como generalmente se describe Estudios electroforéticos de la hemoglobina se han llevado a cabo

El pequeno grupo de personas blancas afectadas de la enfermedad de celulas falciformes ha sido siempre originario del área Mediterránea

Estos hallazgos etnológicos son discutidos desde el punto de vista de una posible existencia de un origen Mediterráneo del fenómeno del sickling

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VIII 3

L'Anémie Drépanocytaire en Grèce

A. GOUTTAS, H. TSEVRENIS, I. FESSAS et P. POUNGOURAS*

L'ANÉMIE drépanocytaire était considérée jusqu'à ces dernières années comme une anémie se rencontrant essentiellement chez les Nègres. Récemment elle a été retrouvée (26 cas à notre connaissance) chez la race blanche. La plupart d'entre eux concernaient des Grecs et des Italiens d'Amérique. C'est en 1940 que le premier cas d'anémie drépanocytaire a été décrit en Grèce par Makrykostas[1] suivi de Zaverdinos[2], Tos, Kondi et Alexandridis[5] et autres[6, 7, 8]. Mais le travail le plus important a été publié par le professeur Choremis et ses collaborateurs en 1951. Ces auteurs ont remarqué que la plupart d'enfants qui présentaient cette particularité anémie étaient originaires d'une même région, Petromagoula située dans la Grèce Centrale. Une large enquête sur place a permis de constater que sur les 6 000 habitants de la région 0,25% présentaient une anémie drépanocytaire, taux qui, selon les auteurs, dépasse celui des Nègres d'Amérique. Nous mêmes avons cherché à découvrir cette anémie chez tous les malades de notre service qui présentaient une anémie avec ou sans ictère. Nous voulons donc rapporter nos résultats et poser en général le problème de l'anémie drépanocytaire en Grèce.

Méthode

Nous avons employé la méthode classique. Asphyxie locale d'un doigt pendant cinq minutes, une goutte de sang posée entre lame et lamelle, enfin paraffine autour de la préparation. Nous avons l'habitude de poser au préalable une goutte de bleu crésyl sur la lame afin de mettre aussi en évidence les réticulocytes drépanocytaires. La lecture est faite ½ heure, 2 heures, 24 h après incubation à 37°.

Nous avons retenu comme positifs tous les cas qui présentaient une falciformisation intense, diffuse et constante sur toutes les préparations et au cours de tous les examens répétés (Photo 1 et 2).

Nous avons éliminé les cas douteux qui présentaient le caractère falciforme soit aux bords des préparations soit en colonies isolées au milieu des erythrocytes normaux (Photo 3). Ces cas nous les considérons comme des artefacts et ne doivent pas être interprétés comme ayant le caractère drépanocytaire vrai. Enfin à notre avis il n'est pas possible d'observer les drépanocytes sur les préparations colorées au May Grunwald Giemsa.

Nous avons été surpris du grand nombre d'anémies drépanocytaires que nous avons trouvé. En effet depuis Janvier 1951 jusqu'à ce jour nos cas se montent à 15. L'âge de ces malades variait de 14 à 60 ans. Le lieu d'origine de

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sickling, suggest that sickling obtained in these cases by the moist stasis method may be of a different origin and character from the African type of the disease

Sickling en Judíos Orientales

Hemos encontrado rasgos de células falciformes (sickle cell trait) entre varios grupos de judíos orientales en quienes la mezcla con sangre negra es muy improbable por varias razones En un grupo de niños pertenecientes a estas comunidades la incidencia de estos rasgos a demostrado ser de un 11%

El fenomeno del sickling encontrado en estos individuos demostro cierta desviacion menor que el comportamiento del sickling en africanos como generalmente se describe Estudios electroforéticos de la hemoglobina se han llevado a cabo

El pequeno grupo de personas blancas afectadas de la enfermedad de células falciformes ha sido siempre originario del área Mediterránea

Estos hallazgos etnologicos son discutidos desde el punto de vista de una posible existencia de un origen Mediterráneo del fenómeno del sickling

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Photo 1 (drépanocytes cas No 10) 2 (drépanocytes cas No 8) et 3 (artefact)

tique certaine et confirmée par les examens de laboratoire Dans les autres cas (No 9) une cardiopathie décompensée avec hépatomégalie Trois cas concernaient des femmes enceintes qui ont presenté leur anémie surtout au terme de leur grossesse Nous avons fait le diagnostic d un quatrieme cas mais il n a pas éte hospitalisé dans notre service Il est a remarquer que trois sur les quatre cas étaient des primipares Le quatrieme cas était une multipare qui accusait les memes symptomes aux cours de chaque grossesse Aucun des enfants n ayant survecu plus de quelques jours a fait penser tout d'abord qu il pouvait s agir

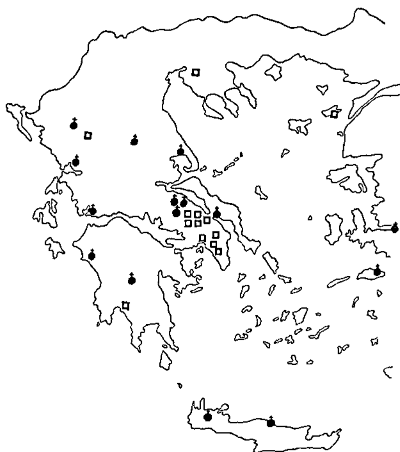


FIG 1 —L anémie drépanocytaire en Grèce Crète etc (● cas personnels □ cas d autres auteurs)

nos cas est tres varié comme on peut le voir sur la carte avec peut etre une certaine prédilection à certains endroits comme l Epire et la Grece Centrale La plupart de nos malades ne souffraient de leur anemie que d une façon passagere et transitoire Certains meconnaissaient completement son existence ayant simplement ressenti à certaines periode de l année une fatigue accompagnée d une paleur et d une légere jaunisse Certains accusaient des douleurs osseuses particulierement intenses

C est brusquement sous l influence d un facteur déclenchant qui dans nos cas était souvent le meme que la crise hemolytiques est installée atteignant des degres divers souvent dramatiques C est a la suite de ces crises de déglobulisation que ces malades ont ete hospitalises dans notre service Toutefois nous soulignons que deux cas avec test drépanocytaire positif présentaient l un la maladie de Hogdkin (cas No 15) l autre une leucose lymphoblastique qui pouvaient à elles seules expliquer l existence de cette anemie hémolytique

Les facteurs declenchants ont été dans nos cas assez voisins pour qu on puisse en tenir compte C est ainsi que dans deux cas il s agissait d une atteinte hépa

TABLEAU 1 — *Les Examens de laboratoire*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age—sexe	18/I	47/M	13/I	[illegible]9/I	[illegible]0/M	[illegible]1/I	30/I	30/I	27/M	45/M	2[illegible]/M	10/I	41/I	3[illegible]/M	[illegible]/M
Globules rouges millions/m³	3 2	3 1	4	2 2	3 2	[illegible]	3 1	2 [illegible]	[illegible] 8	2 1	[illegible] 9	1 1	2 [illegible]	2 [illegible]	1 [illegible]
Hématocrite	31	31	31	[illegible]3	30	20	31	[illegible]	[illegible]7	[illegible]7	[illegible]	13	20	21	16
Hémoglobine gr % c c	8 [illegible]	8 [illegible]	7	4 [illegible]	[illegible] 5	4	8 [illegible]	[illegible]	9	8	9 [illegible]	3 [illegible]	[illegible]	8	4
M C V μ³	91	90	[illegible]8	104	81	98	100	96	100	12[illegible]	110	70	68	81	108
M C H γγ	[illegible]	3[illegible]	17	20	1[illegible]	20	29	22	30	33	33	-	17	33	2[illegible]
M C C %	[illegible]	[illegible]	27	1	1[illegible]	20	2[illegible]	23	31	30	33	[illegible]	2[illegible]	3[illegible]	[illegible]
Réticulocytes %	8	7	0				4	7 [illegible]	8	[illegible]0	11		9		14
Erythroblastes/m	–	–		13000		80000				[illegible]8000	2800	3600		400	6[illegible]0
Globules blancs/m³	2[illegible]00	7100	11000	6000	6[illegible]00	1[illegible]000	8000	6200	[illegible]100	30000	13100	[illegible]1000	1[illegible]000	6000	14000
Myélogramme erythro %	[illegible]0	1[illegible]					41	4						63 [illegible]	
Résistance globulaire	4 [illegible]	3 9[illegible]	3 9[illegible]	3 7[illegible]	4 5	3 5	3 [illegible]	5 [illegible]	3	4	3		4	3 [illegible]	
	1 [illegible]	1	1	1 7[illegible]	[illegible] 7[illegible]	1 5	[illegible]	3 [illegible]	1	1 1[illegible]	1		2 [illegible]	1	
Test Coombs				–				–							
Test Dacie Caroli				–				–							
Test Crosby			–					–					–		
Test Ham			–					–					–		
Alcali résistance %										6 [illegible]	[illegible] 0				
Sédimentation	6			[illegible]0	1[illegible]	2	10	30				130	13[illegible]		
Bilirubine directe	–	+				–	–	–	–	+	–		+		–
Bilirubine indirecte en mg%	11 [illegible]	3				1 [illegible]	0 9[illegible]	0 20	1 7[illegible]	2[illegible] 7	0 [illegible]		1 8		0 [illegible]

d une incompatibilité Rh ou A B O Toutes nos malades étaient Rh positifs et il n y avait aucun anticorps dans leur serum

Il semble donc logique connaissant le role de la grossesse sur le foie de considérer que dans les cas precités l'atteinte hepatique jouerait en définitive le role d épine irritative sans pouvoir il est vrai expliquer le mécanisme d action Enfin une leucose lymphoblastique (cas No 12) avec forme hépatomegalique vient encore plaider en faveur de notre hypothèse On peut rapprocher les travaux de Hyman et Southworth[10] sur l'association d anémies hemolytiques avec atteinte hepatique mais avec cette différence que dans ce travail si interessant, il s agit surtout d anémies hemolytiques acquises

Signes Cliniques

Sans vouloir nous étendre sur la symptomatologie clinique de cette anémie nous voudrions toutefois rapporter quelques points qui nous ont paru interessants

(a) La plupart de nos malades présentaient une rate a peine palpable Dans deux cas (No 1 et 9) elle atteignait l ombilic Dans un cas (No 10) elle était absente En effet la vérification autopsique n a pas permis de reveler la moindre trace de rate

(b) Les stigmates osseux radiologiques n ont pas ete trouvé dans tous nos cas (Photo 4 et 5) De meme nous n avons pas observe aussi fréquemment que le prétendent les classiques les ulceres de jambe En effet dans notre série un seul malade presentait des cicatrices comme sequelles de cette lésion cutanee Les examens de Laboratoire sont representes sur le Tableau 1 De ce tableau il ressort

1 Presque tous nos malades présentaient une anemie dont le degré variait entre 1 500 000 et 3 500 000 Cette anémie etait en général normocytaire avec

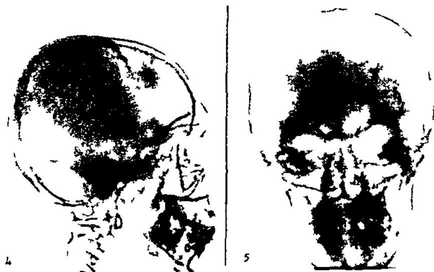


Photo 4 et 5 (cas No 11)

Il résulte donc que l'anémie drépanocytaire est plus fréquente que l'anémie type Cooley tout au moins chez l'adulte. Quant à la fréquence générale de l'anémie falciforme notre statistique portant sur un total de 1800 malades hospitalisés dans notre service (110 lits) nous donne un taux de fréquence atteignant les 15 sur 1800, c'est-à-dire 0,80 %. Il est vrai que notre clinique reçoit une grande partie des cas hématologiques.

Quant aux sujets présentant le trait drépanocytaire sans aucune manifestation hémolytique nous l'avons cherché chez les ascendants et les parents de nos malades. Nous l'avons trouvé presque toujours positif. Nous n'avons pu étendre nos investigations sur une grande échelle pour pouvoir donner un chiffre approximatif de la fréquence de ce test en Grèce.

Toutefois en se basant sur nos résultats nous pouvons conclure que les chiffres de 19 % donné par Caminopetros nous paraît nettement exagéré. Par contre nos résultats concordent avec ceux de Choremis et ses collaborateurs. En effet ces auteurs ayant cherché le test drépanocytaire chez 1016 habitants de Petroma goula d'âge variant de quelques mois à 80 ans l'ont trouvé positif dans 13 % à 14 % des cas. *L'anémie drépanocytaire se rencontre dans toute la Grèce comme* le montre notre carte et atteint les îles les plus éloignées, Samos, Crète.

Il y a certainement des régions où cette anémie se rencontre plus volontiers, mais il faut considérer ce problème sous un autre angle, c'est-à-dire que les habitants de ces régions ne se déplacent que rarement et favorisent ainsi les mariages consanguins.

Il est hors de doute que le fait que l'anémie drépanocytaire est rencontrée sur toute la Grèce exclue toute hypothèse de métissage avec les Nègres. En effet des incursions des Nègres n'ont jamais été rapportées dans le passé. Ceci d'ailleurs n'empêche pas le métissage sporadique par des esclaves amenés par des envahisseurs, mais cette hypothèse n'est point valable si on considère le haut pourcentage du test drépanocytaire.

La seule incursion vérifiée est celle des Egyptiens sous Imbraim Pacha dans le Péloponèse en 1827. De toute façon les quelques rares esclaves Nègres qui ont pu rester après l'expulsion de l'envahisseur ne peuvent pas expliquer cette dissémination de l'anémie drépanocytaire. Il semble donc logique d'exclure tout métissage possible avec les Nègres.

Un problème important est aussi les rapports de l'anémie drépanocytaire avec l'anémie de Cooley. Nous savons en effet que l'anémie de Cooley est aussi très fréquente en Grèce. Des nombreux signes communs relient ces deux anémies. En effet toutes les deux A. D. et A. C. sont héréditaires et familiales. Elles présentent les mêmes signes osseux, des ulcères de jambe; l'augmentation de la résistance globulaire aux solutions hypotoniques est commune, mais nous voulons insister surtout sur des points qui nous paraissent intéressants.

1. *Le facies* des anémiques nous paraît très différent. Dans l'anémie drépanocytaire le faciès est plutôt allongé, les malades possèdent des traits fins, ce sont en général des longilignes (Photo 8) en opposition aux anémiques type Cooley qui sont plutôt tassés avec facies à tendance mongoloïde et qui se rapproche plus de l'anémie sphérocytaire type Minkowski Chauffard.



PHOTO 6 (cas No 6) et 7 (cas No 10)

tendance macrocytaire Elle etait toujours hypochrome comme le montre la valeur MCV

2 Erythroblastose intense au cours des crises hemolytiques parfois atteignant des chiffres élevés 70 000 (Photo 6 et 7)

3 La résistance globulaire etait nettement augmentée meme d apres la methode de Varadi

4 Tous les autres tests d hemolyse que nous avons pratiqué se sont revelés negatifs

Dans un cas (No 13) qui présentait une resistance globulaire diminuee le test de Dacie Caroli nous a permis de rectifier ce diagnostic En effet ce test s est revelé normal

5 La leucocytose était presque toujours normale parfois augmentée avec formule leucémoide au cours des crises d hémolyse Certains cas avec forte splenomegalie s accompagnaient de leucopenie

6 L etude morphologique des hématies nous a permis de constater que les réticulocytes drépanocytaires existent atteignant des chiffres de 10 à 20% Nous avons aussi constaté souvent la présence d un tres grand nombre d hématies à cible atteignant dans certains cas des taux de 70 à 80% des hematies

7 Le myélogramme revele une réaction érythroblastique intense en rapport avec l anémie

8 Enfin phenomene curieux constaté par des nombreux auteurs la sédimentation globulaire est presque normale Dans les cas ou elle est augmentée (No 12 et 13) il s agissait d une leucose et d une maladie d Hogdkin

Une simple statistique des maladies hemolytiques constitutionnelles entrés dans notre service nous fait constater que l anémie drépanocytaire tient une place prépondérante

En effet sur les 23 cas d anemies hémolytiques les 15 étaient drépanocytaires les 3 type Cooley et 1 du type Minkowski Chauffard

Nous mêmes avons trouvé des résultats sensiblement identiques avec les auteurs précités.

Toutefois la présence de l'hémoglobine Alcalo-résistante dans d'autres anémies fait constaté également par nous-mêmes enlève tout caractère spécifique à la méthode de Singer tout en gardant une valeur certaine comme moyen de diagnostic positif.

Pauling et ses collaborateurs étudiant l'hémoglobine avec l'électrophorèse ont pu démontrer l'existence d'une hémoglobine spécifique de l'anémie drépanocytaire, tant dans sa forme hémolytique que chez les porteurs du trait. Malheureusement nous n'avons pu appliquer cette méthode si précieuse qui nous aurait permis de différencier avec certitude l'anémie drépanocytaire de l'anémie type Cooley.

Les familles de nos malades habitant loin d'Athènes il a été difficile de faire une étude complète de leur arbre généalogique qui nous aurait permis de rechercher la possibilité de coexistence ou non de deux maladies.

A notre avis le fait que l'anémie type Cooley est très rare chez l'adulte alors que l'anémie drépanocytaire est très fréquente plaiderait en faveur de la non coexistence de deux maladies.

En effet, on sait que l'anémie Cooley dépasse rarement la 12me année. Il serait donc paradoxal que les quelques rares cas arrivés à l'âge adulte soient associés à l'anémie drépanocytaire.

Nous pensons donc avoir ainsi contribué surtout à abolir l'idée de l'existence de l'anémie drépanocytaire essentiellement chez les Nègres et montrer que cette anémie est très fréquente en Grèce et probablement plus fréquente que l'anémie de Cooley, tout au moins chez l'adulte.

Résumé

Les auteurs apportent leur statistique sur les anémies drépanocytaires, une étude de principaux signes cliniques et des examens de Laboratoire et concluent que cette anémie est très fréquente en Grèce et probablement plus fréquente que l'anémie type Cooley, tout au moins chez l'adulte.

Sickle Cell Anemia in Greece

In 1940 Makrykostas described the first case of sickle cell anemia in Greece. Subsequently Zaverdinos (1950), Kominos and Bakalos (1950), Choremis and col. (1951) etc. described similar cases.

Since January 1951, at which time we began our observations, we have studied 12 cases of sickle cell anemia in a total of 21 cases of constitutional hemolytic anemias which entered our Clinic (numbering 110 beds).

As our patients originate from various parts of Greece and as no cross-breeding with the Negro race is mentioned in Greek history, we believe that Negro admixture can be excluded.

The similarity of sickle cell anemia to Cooley's anemia presents two interesting problems, i.e. the coexistence of the two anemias or the transformation of the one into the other.

We believe that only the settling of the quality of hemoglobin (either by electrophoresis or by any other method) will allow us to clear the problem of the duality of these two anemias.

Our observations reveal also that sickle cell anemia is much more frequent than Cooley's anemia in our country, at least among adults.



PHOTO 8 (cas No 8 et cas No 7)

2 *En examinant une lame de sang colorec* du May Crunwald Giemsa on con state que dans l anemie drepanocytaire typique il existe des preparations avec des rares hematies a cible (Photo 9) Par contre il existe des cas ou les sang peri pherique coloré montre une abondance d hématies a cible (Photo 10) Ceci vient encore poser le probleme de la parenté ou de la simple coexistence de deux mala dies

Probleme difficile il est vrai a resoudre avec seulement les caracteres morpho logiques

Recemment Singer et Chernoff[8] étudiant l hémoglobine resistante a l Alcali ont pu demontrer l existence de celle ci dans les cas d anemies de Cooley et d anémies drépanocytaires

En Crece Mlle Zannou[11] dans le service du professeur Choremis a pu appliquer la methode de Singer dans plusieurs cas d anémies drépanocytaires et d anémies type Cooley Elle a confirme les resultats de Singer et Chernoff Elle a pu en plus observer une legère différence entre les deux anémies c à d que le taux de cette hémoglobine était legèrement inferieure dans les anémies drépanocytaires

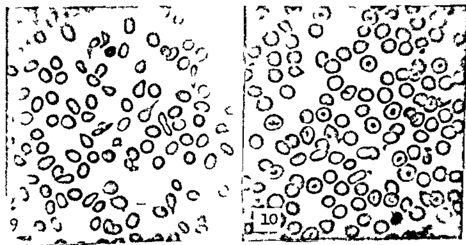


PHOTO 9 (cas No 1) et 10 (cas No 2)

ción del que lo realiza. Hay que destacar que en Chile hasta 1941 el diagnóstico de lupus eritematoso diseminado prácticamente era desconocido y fue Armas Cruz el primero que se preocupó de identificar esta enfermedad y en 1950 con Hamecker publicó su experiencia basada en 15 casos. Ese mismo año los autores efectuaron con éxito la búsqueda de las células del L. E. en la sangre periférica y desde entonces hasta el momento de esta presentación han podido confirmar el diagnóstico de 26 nuevos casos en el breve período de 2 años, esto gracias a que la búsqueda de las células del L. E. permite catalogar actualmente muchos cuadros que antes eran etiquetados como artritis reumatoidea, enfermedad reumática, anemias hemolíticas de origen desconocido, etc. Conviene señalar que esta búsqueda debe realizarse siempre que los síntomas clínicos hagan sospechar fuertemente el diagnóstico de lupus eritematoso diseminado, pues algunos de los fenómenos que se describirán más adelante pueden encontrarse aislados o en forma atípica en algunos cuadros que no corresponden a esta enfermedad.

Hargraves, Richmond y Morton en 1948 describieron la célula del L. E. en la médula ósea de enfermos de lupus eritematoso agudo diseminado. Esta célula sería habitualmente un granulocito neutrófilo que además de su núcleo lobulado habitual presentaría en su interior una masa redondeada que semeja un segundo núcleo. Haserick y Sundberg corroboraron este hallazgo y posteriormente Sundberg y Lick demostraron la existencia de células del L. E. en la sangre periférica. Desde entonces numerosas publicaciones han demostrado la efectividad de estas observaciones y es así como además de las presentadas por autores norteamericanos han aparecido publicaciones de autores argentinos y franceses.

Hoy se estima que el proceso que llevaría a la formación de las células del L. E. se realizaría en 2 fases: en la primera se produciría una rápida autolisis del núcleo de algunos granulocitos neutrófilos y en la segunda se produciría la fagocitosis del núcleo autolizado por otro leucocito en buen estado de conservación, sea éste un granulocito neutrófilo o eosinófilo o un monocito.

Para la producción del fenómeno se requerirían 2 componentes: uno plasmático, contenido en la fracción gamma globulina del plasma de los enfermos de lupus eritematoso diseminado y que ha sido separado electroforéticamente. Este factor plasmático sería el causante de la autolisis del núcleo de algunos granulocitos neutrófilos, tal vez por la depolimerización del ácido desoxirribonucleico. El otro componente sería celular y estaría representado por los granulocitos neutrófilos intactos que fagocitan el núcleo autolizado. El componente decisivo es el plasmático, ya que se ha demostrado que mezclando plasma de enfermos de Lupus Eritematoso Diseminado con concentrados leucocitarios normales obtenidos de médula ósea o de sangre periférica el fenómeno se reproduce en igual forma que con los granulocitos del propio enfermo.

Con respecto a la naturaleza del material fagocitado no hay ninguna duda que éste es de origen nuclear. Ya Hargraves sostuvo que era la autolisis nuclear de un granulocito neutrófilo que daba la reacción de Feulgen positiva. Posteriormente se ha dicho que sería el núcleo de un linfocito o de origen trombocítico. Los estudios de Lee, Rohn y Bond y nuestras propias observaciones demuestran que se trata de la fagocitosis de una masa nuclear procedente de un granulocito neutrófilo. En un intento de dilucidar este problema Moyer y Fisher

Anemia a Células Falciformes en Grecia

En 1940 Makrykostas describe el primer caso de anemia de células falciformes en Grecia Más tarde Zaverdinos (1950) Kominos y Bakalos (1950) Choremis y colaboradores (1951) describen casos similares

Desde enero de 1951 en que comenzamos nuestras observaciones encontramos doce casos de *anemia a células falciformes* en un total de 21 casos de anemias hemolíticas constitucionales que entraron en nuestra clínica

Como nuestros pacientes proceden de varias partes de Grecia y no hay *antecedentes de* cruza con negro, creemos que esta hipótesis puede ser descartada La similitud de la drepanocitemia y la anemia de Cooley plantea dos interesantes problemas la *coexistencia de* las dos anemias o la transformación de una en otra

Creemos que solo el establecimiento de la calidad de la hemoglobina (sea por electroforesis u *otro método*) nos permitirá aclarar el problema de la dualidad o identidad de ambas anemias

Nuestras observaciones revelan también que la anemia a células falciformes es mucho *más* frecuente en nuestro país que la anemia de Cooley por lo menos entre los adultos

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VIII 4

Células del Lupus Eritematoso

GERMAN DUCACH y JUAN GRANIC*

El HALLAZGO de las células del lupus eritematoso (L E) ha permitido que una afección cuyo diagnóstico solo podía hacerse por presunción actualmente se llegue a el en forma precisa por un simple estudio citológico que no requiere instrumental complicado reactivos químicos o una gran prepara

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Uno de los puntos más discutidos en relación a los fenómenos del L. E. es el que se refiere a su especificidad. Prácticamente todos los que han escrito al respecto están de acuerdo que estas células sólo se encuentran en el lupus eritematoso diseminado. Se han descrito algunos casos falsamente positivos, pero en general ellos se refieren a falsas células de lupus por tener caracteres atípicos o bien a fenómenos incompletos. Haserick lo encontró sin excepción en 23 casos estudiados y no apareció en ningún caso de enfermedades del mesénquima en que fueron buscadas. Hargraves además del lupus sólo encontró la aparición del fenómeno en un caso de mieloma múltiple. Vonder Heide vió células del L. E. en un enfermo con leucemia. Berman y cols. dicen haber encontrado este fenómeno en 6 casos que no eran de lupus, pero agregan que el número de células L. E. era muy inferior al que se encuentra en el lupus. Estos 6 casos corresponden a los siguientes diagnósticos: anemia perniciosa, dermatitis herpetiforme, lupus discoídeo crónico, lupus eritematoso crónico y una probable enfermedad del mesénquima. Estos mismos autores afirman que una concentración de 10 células L. E. por 500 baciliformes o granulocitos neutrófilos en la médula ósea sería suficiente para asegurar el diagnóstico de L. E.

Técnicas Empleadas

En el presente trabajo los fenómenos del L. E. se buscaron en la sangre periférica o en la médula ósea o en ambas. Hemos preferido siempre su búsqueda en la sangre periférica ya que su porcentaje de positividad es casi igual al de la médula ósea y evita la punción esternal que es siempre resistida por los enfermos sobre todo cuando es necesario repetir los exámenes.

Para la investigación en la sangre periférica se extraen con una jeringa seca 5 cc. de sangre de una vena del pliegue del codo y se mezclan con la mezcla oxalatada de Wintrobe. Se deja la sangre a la temperatura ordinaria durante 1 a 2 horas y se vierte en un tubo de hemolisis centrifugándose a 1.500 revoluciones durante 5 minutos. Posteriormente se aspira el plasma con una pipeta el cual se guarda para la prueba indirecta. Luego se aspira la capa de leucocitos y plaquetas (buffy coat) y se hacen frotis en portaobjetos. Habitualmente el aspecto de esta capa de leucocitos es similar al de la médula ósea obtenida por punción, de color amarillo y grumoso. Los frotis se tiñen con colorante de Wright o con May Grunwald Giemsa. Con este procedimiento se obtiene un concentrado de leucocitos en una proporción de más o menos un leucocito por 10 eritrocitos. La mezcla de plaquetas es variable, a veces son muy escasas, pero en otras oportunidades son tan numerosas que incluso dificultan el examen. Para la búsqueda en la médula se obtienen 3 cc. de sangre medular por punción del cuerpo del esternón y se procede como con la sangre. Las pruebas indirectas se efectuaron mezclando plasma de enfermos de lupus con concentrados de leucocitos compatibles según el grupo sanguíneo obtenidos en la forma arriba indicada. La gamma globulina se obtuvo por separación electroforética.

Conviene señalar que si bien es cierto que la búsqueda es relativamente sencilla, pues se encuentran con facilidad todos los fenómenos del L. E., otras veces ella es sumamente tediosa y larga, exigiendo la revisión de varias láminas. En este sentido no estamos de acuerdo con Berman y cols. acerca del hecho que se

incubaron plasma de enfermos de lupus eritematoso con concentrados de neutrofilos frescos concentrados de sangre envejecida en el banco de sangre que segun ellos contiene casi exclusivamente linfocitos o sus nucleos y concentrados de linfocitos obtenidos de sangre de una leucemia linfatica cronica y observaron que no habia formacion de celulas del L E en las incubaciones de neutrofilo que habia una regular cantidad en las incubaciones de sangre envejecida y una gran cantidad en las incubaciones con concentrados de sangre de leucemia linfatica cronica indicando con esto, que la masa fagocitada seria de origen linfocítico Lee y cols haciendo incubaciones de plasma de enfermos de L E con concentrados obtenidos de *enfermos de leucemia mieloide cronica y leucemia* linfoide cronica concluyen que incubando granulocitos neutrofilos unicamente el numero de celulas del L E es escaso que incubando linfocitos unicamente no se produjeron celulas del L E pero que cuando incubaron mezclas de granulocitos y linfocitos se produjeron gran cantidad de celulas del L E e incluso pudieron comprobar fagocitosis de linfocitos intactos por granulocito maduros e inmaduros Estos autores concluyen que la masa fagocitada de la celula del L E puede ser de origen linfocitico o de origen granulocitico

Se han empleado numerosos métodos para provocar la induccion de los fenomenos del L E En un comienzo Hargraves los encontro en concentrados de medula osea mezclados con heparina Se creyo entonces que solo se encontraban en la medula osea y que era necesario la presencia de un anticoagulante que ademas de la heparina podía ser el oxalato de potasio y de amonio y el citrato de sodio *Tambien se creyo que la centrifugacion del material era necesaria para* así poner en contacto en forma mas completa a los componentes necesarios para la produccion de estos fenomenos Posteriormente se han encontrado en la sangre periferica en concentrados leucocitarios obtenidos de sangre mezclada con anticoagulantes y ultimamente ya se ha observado que el anticoagulante no es necesario ya que se han encontrado en la sangre coagulada en la sangre desfibrinada y en la sangre obtenida en material revestido de silicio Al parecer el factor decisivo para la produccion del fenomeno es el tiempo que varia entre 2 minutos y 20 minutos Se cree que ya a los 40 minutos el fenomeno es total Nosotros hemos visto aparicion de celulas tipicas del L E en *frotis efectuados* ya a los 5 minutos de extraida la sangre Diversos autores se han preocupado de buscar las celulas del L E en frotis corrientes preparados directamente con la sangre del pulpejo del dedo sus resultados han sido siempre negativos con excepcion de una observacion de Penalvez que refiere haber encontrado tipicas celulas del L E en la sangre del pulpejo del dedo en un caso de L E diseminado agudo y anemia hemolitica aguda adquirida con anticuerpos Es probable que estos fenomenos que solo se observan in vitro ocurran en el organismo de los enfermos de L E y que corresponden a los acumulos de corpusculos que se tinen con la hematoxilina en las vegetaciones valvulares de la enfermedad de Libman Sacks y que ya fueron demostrados por Gross en 1932 por Ginzler y Fox en ganglios linfaticos necroticos de un caso de L E y también en los glomerulos del riñon Estas masas estan formadas por acumulos de nucleos de *celulas del tejido conjuntivo* incluso granulocitos neutrofilos y dan la reaccion de Feulgen positiva

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En el presente trabajo los fenómenos del L E se buscaron en la sangre periférica o en la médula ósea o en ambas Hemos preferido siempre su búsqueda en la sangre periférica ya que su porcentaje de positividad es casi igual al de la médula ósea y evita la punción esternal que es siempre resistida por los enfermos sobre todo cuando es necesario repetir los exámenes

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pueda hacer una apreciación cuantitativa de las células del L E con un mínimo de 2% para asegurar el diagnóstico Los fenómenos del L E se distribuyen en forma irregular y en algunos puntos de la preparación son mucho más abundantes que en otros Es por esto que este examen debe hacerse siempre con conocimiento de la sintomatología clínica del paciente con objeto de hacer una búsqueda lo más completa posible cuando hay sospecha de un diagnóstico positivo En estos casos con fuerte presunción clínica en los que repetidas investigaciones en la sangre periférica resultan negativas, recomendamos la búsqueda en la médula ósea ya que en 2 de nuestros enfermos las encontramos positivas en la médula ósea con absoluta negatividad en la sangre periférica

Descripción de los Fenómenos del L E

Los fenómenos citológicos del L E pueden describirse en la siguiente forma

1 La célula del L E (Fig 1 y 2) es habitualmente un polinuclear neutrófilo

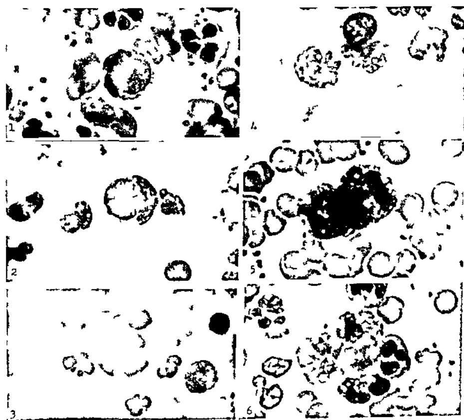


Fig 1 6

Fig 1 —Célula del lupus típica
Fig 2 —Célula del lupus típica La masa fagocitada conserva cierta estructura
Fig 3 —Célula del lupus que presenta una masa doble en su interior
Fig 4 —Monocito con una masa incluída
Fig 5 —Roseta formada por [illegible] granulocitos neutrófilos
Fig 6 —Roseta formada por 4 granulocitos neutrófilos

que contiene una masa incluida que ocupa casi todo el protoplasma y que rechaza el nucleo hacia un costado a veces se observa una muy pequeña capa de protoplasma con algunas granulaciones alrededor de esta masa La masa incluída es habitualmente unica pero puede ser doble (Fig 3) de forma redonda amorfa y de color violeta palido Puede ser granulocito o bacıliforme neutrofilo o bien un granulocito eosinofilo o bien un monocito (Fig 4)

2 La roseta en la cual 2 o mas granulocitos neutrofilos han fagocitado una misma masa (Fig 5 6 7 y 8) a veces entre los granulocitos puede haber algun eosinofilo (Fig 9) otras veces en una misma roseta pueden haber varias células de lupus (Figs 10 y 11)

3 Los grumos de granulocitos neutrofilos en ellos no se encuentra una masa central (Figs 12 y 13)

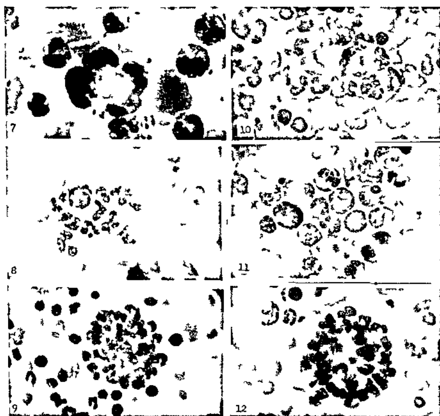


Fig 7 12

Fig 7 —Roseta

Fig 8 —Roseta formada por un gran numero de granulocitos neutrofilos

Fig 9 —Roseta en la que forma parte un granulocito eosinófilo

Fig 10 —Roseta con varios granulocitos neutrofilos

Fig 11 —Roseta con varias celulas del L E

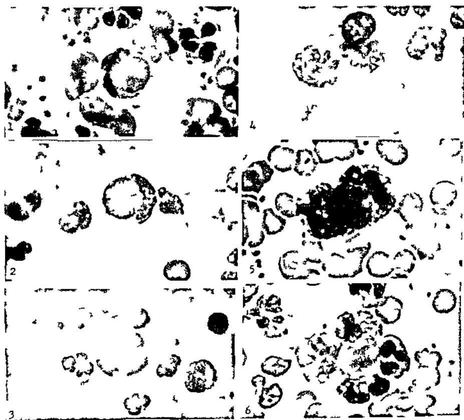
Fig 12 —Grumo de granulocitos neutrófilos

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Fig 6 —Roseta formada por 4 granulocitos neutrófilos

Resultados de Nuestras Observaciones

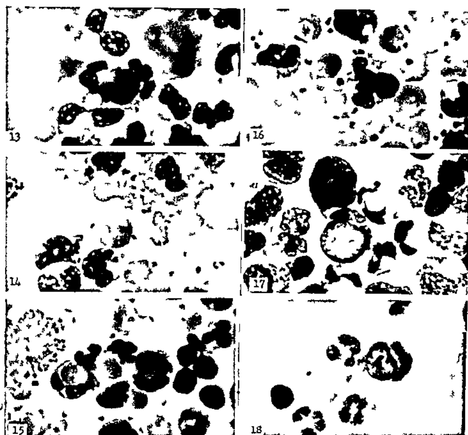
Se han estudiado 26 casos de lupus eritematoso diseminado y 2 casos de lupus discoideo fijo

De los 26 casos de L E Diseminado 20 eran del sexo femenino y 6 del sexo masculino y sus edades fluctuaron entre 14 y 62 años

Los fenómenos del L E resultaron positivos en todos los enfermos En 24 se encontraron en la sangre periférica y en los 2 restantes en los que esta busqueda fue negativa se encontraron positivos en la medula osea En 2 de estos casos en los que la busqueda se hizo simultaneamente en la sangre y en la medula osea los fenomenos fueron mas abundantes en la sangre El fenomeno completo o sea presencia de celulas del L E rosetas y aglutinacion de los leucocitos se encontro en 21 casos y fué marcado en 13 A este respecto deseamos señalar que las infecciones intercurrentes exageran considerablemente los fenomenos del L E Es así como en 3 enfermos de nuestra serie 2 de ellos en pleno tratamiento con cortisona y que tuvieron alguna infeccion intercurrente pudimos observar un notable aumento en el numero de células del L E y de las rosetas como si la infeccion hubiese exagerado los mecanismos productores del fenomeno Estas infecciones fueron una neumonia neumococica una erisipela y una sepsis por Salmonella cuyo punto de partida fue la vesicula biliar No observamos disminucion de los fenomenos en los enfermos moribundos

Respecto a los tratamientos podemos señalar que 22 fueron sometidos a tratamiento con cortisona Las dosis en general fueron bajas no sobrepasando los 200 mgrs diarios y muchos por razones economicas suspendieron el tratamiento En 4 enfermos se observo una franca disminucion de los fenomenos del L E a tal punto de encontrar solo ocasionalmente una célula del L E despues de una prolongada busqueda en 6 desaparecieron en forma total coincidiendo ésto con dosis elevadas y prolongadas de cortisona En 3 de estos casos observamos la aparicion de un fenomeno celular no descrito y consistente en la aparicion de granulocitos neutrofilos que estan rodeados por un anillo de material nuclear (Figs 19 20 y 21) y que nosotros hemos denominado celulas del L E al revés o invertidas Este podria ser el resto de un monocito que hubiera fagocitado al granulocito como puede verse en la Fig 22 La aparicion de este tipo de celulas la hemos visto tambien en un caso de leucemia mieloide cronica en el que ademas encontramos escasas celulas L E atípicas En 5 enfermos los fenomenos persistieron igual que antes del tratamiento aun con mejoria clinica de los enfermos Al respecto los datos bibliograficos son contradictorios ya que algunos sostienen que los fenomenos desaparecen totalmente con el tratamiento siempre que se usen dosis altas y otros so tienen que los fenomenos persisten a pesar del tratamiento Este es un punto que esta en estudio y debera resolverse en el futuro cuando los enfermos hayan sido estudiados por periodos mas prolongados de tiempo En los 7 enfermos restantes no se pudo saber lo ocurrido con los fenomenos del L E pues abandonaron el Hospital para hacerse tratamiento en su domicilio y no volvieron a controlarse

Con el plasma de 5 enfermos se hizo la prueba indirecta resultando en todos



Figs 13 18

Fig 13 —Grumo de granulocitos neutrófilos
Fig 14 —Masa nuclear libre
Fig 15 —Masa nuclear libre
Fig 16 —Célula L E atípica de un caso de pericarditis Tbc
Fig 17 —Célula atípica en la médula de un caso de cáncer del páncreas
Fig 18 —Célula atípica en un caso de lupus crónico discoideo

4 *Las masas nucleares libres que corresponden a masas iguales a las que se* observan en el interior de los granulocitos pero que en estos casos no han sido fagocitados (Figs 14 y 15)

5 Granulocitos o baciliformes neutrófilos con su núcleo hinchado próximo a perder su estructura pero conservando el protoplasma intacto (Fig 25 y 26)

De estos hechos los más importantes y útiles para el diagnóstico son los dos primeros pues los otros tres no son específicos ni constantes

Las células atípicas corresponden a la tart cell de Hargraves y pueden describirse como un granulocito neutrófilo que tiene una masa fagocitada en su interior de tamaño pequeño con estructura nuclear conservada y contorno muy bien delimitado (Fig 16 17 y 18)

Resultados de Nuestras Observaciones

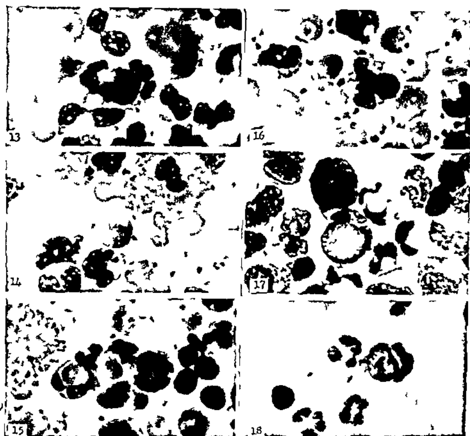
Se han estudiado 26 casos de lupus eritematoso diseminado y 2 casos de lupus discoideo fijo

De los 26 casos de L E Diseminado 20 eran del sexo femenino y 6 del sexo masculino y sus edades fluctuaron entre 14 y 62 años

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Figs 13-18

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Las células atípicas corresponden a la "tart cell" de Hargraves y pueden describirse como un granulocito neutrófilo que tiene una masa fagocitada en su interior, de tamaño pequeño con estructura nuclear conservada y contorno muy bien delimitado (Fig. 16, 17 y 18).

Con objeto de estudiar la especificidad de estos fenomenos procedimos a estudiarlos en otras afecciones y es así como realizamos su investigacion en 20 casos de artritis reumatoidea en 10 casos de enfermedad reumatica activa en 6 casos de leucemias agudas y cronicas en 3 casos de mieloma multiple y un caso de periarteritis nudosa un caso de esclerodermia un caso de pericarditis tuberculosa un caso de enfermedad de Hodgkin y un caso de cancer del pancreas En ninguno de estos casos se encontraron los fenomenos tipicos del L E En 5 casos de artritis reumatoidea se encontro aglutinacion de los leucocitos en un caso de este mismo diagnostico se encontraron vacuolas en el interior de los granulocitos neutrofilos (Fig 23) y celulas atípicas (tart cells) se observaron en los casos de pericarditis tuberculosa en la enfermedad de Hodgkin en la esclerodermia y en el caso del cancer del pancreas En todos estos casos en que se encontraron estas celulas atípicas ellas se encontraron en numero muy escaso

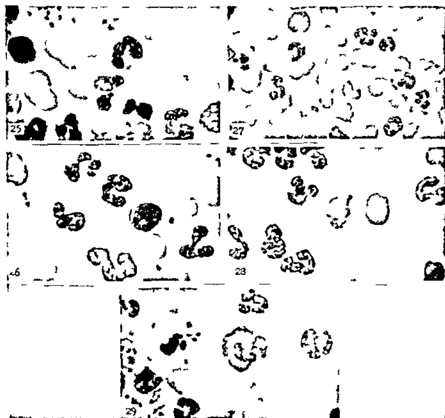
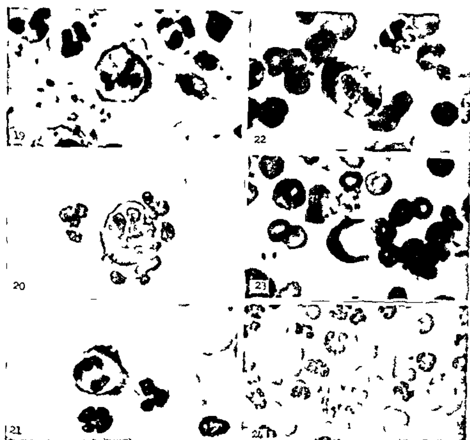


FIG 25–29

FIG 25 —Granulocitos neutrofilos con el nucleo hinchado
FIG 26 —Autólisis nuclear de un granulocito neutrofilo que aun conserva el protoplasma
FIG 27 —Autolisis nuclear que va hacia la formacion de una masa libre
FIG 28 —Granulocito neutrofilo en el momento de fagocitar una masa libre
FIG 29 —Granulocito neutrofilo fagocitando una masa libre



FIGS 19-24

FIG 19 — Célula L E invertida
FIG 20 — Célula L E invertida
FIG 21 — Célula L E invertida
FIG 22 —Granulocitos neutrófilos fagocitados por un monocito
FIG 23 —Granulocito neutrófilo con una vacuola en su interior
FIG 24 —Granulocitos neutrófilos con el núcleo hinchado

los casos positiva En un caso la prueba indirecta efectuada con leucocitos de un enfermo que sufría de un cuadro infeccioso no precisado mostró ser mucho más positiva que con los leucocitos del propio enfermo a tal punto que la mayor parte de los granulocitos eran células del L E La gamma globulina de 2 enfermos separada electroforéticamente y mezclada con leucocitos de una persona normal mostró la producción de típicas células del L E

De nuestros enfermos 5 fallecieron habiéndose practicado la autopsia en 3 con confirmación del diagnóstico

En los 2 enfermos de lupus discoideo fijo en los que se practicó la búsqueda de los fenómenos del L E se encontraron células atípicas (tart cell) en escaso número pero sin estar acompañadas de rosetas ni de grumos de leucocitos (Fig 18)

Muy sugerente es la correlacion de los fenomenos citologicos con los acumulos de *corpusculos que se tinen con la hematoxilina lo cual hace suponer que estos* fenómenos ocurririan in vivo en el organismo de los pacientes de Lupus Eritematoso Diseminado

Conclusiones

1 *Se investigan los fenómenos del L E en 26 casos de esta enfermedad* encontrandose en la sangre periférica o en la medula osea de todos ellos presencia de células del L E rosetas y aglutinacion de los granulocitos neutrofilos

2 *Las células del L E son especificas de la enfermedad y su hallazgo basta* para confirmar un diagnostico siempre que exista la sintomatologia clinica propia de la enfermedad

3 *Las células atipicas del L E se presentan en una serie de cuadros caquectizantes pero sus características morfologicas su escaso numero y la falta de los otros fenomenos del L E hacen que su diferenciacion sea perfectamente posible*

4 Las infecciones intercurrentes exageran considerablemente los fenomenos del L E

5 *La cortisona produce en la mayor parte de los casos disminucion y hasta* desaparicion de las células del L E y es muy probable que su persistencia dependa de una dosificacion insuficiente

6 Los estudios seriados de muestras *recién extraídas demuestran que el material* fagocitado proviene de la autolisis de otro granulocito neutrofilo

Cells of Lupus Erythematosus

The authors present *photomicrographs of 30 cases of lupus erythematosus studied in* the last 2 years representing 28 acute or subacute cases and 2 chronic ones

The phenomenon of lupus erythematosus is described (1) the lupus cell (2) the rosette (3) the agglutination of neutrophil granulocytes The fact that the lupus cell has been observed in neutrophil granulocytes eosinophils and also in *monocytes* is mentioned A special abnormality of the lupus cell which appears in patients treated with cortisone will be described

The authors refer to the specificity lupus erythematosus cells found in peripheral blood as well as in the bone marrow and they show microphotographs of false lupus cells found in other affections

y una vez que se conoce su morfología, su diferenciación con la célula del L E no ofrece grandes dificultades

Con objeto de estudiar las distintas etapas de la formacion de las células del L E practicamos una extension de concentrado leucocitario 5 minutos despues de extraída la sangre de un caso que tenía gran actividad productora de los fenomenos del L E por haberse agregado una erisipela En esta preparacion pudimos seguir perfectamente el fenomeno tal como lo han esquematizado Rohn y Bond que efectuaron su estudio con coloraciones vitales Se pudo observar primero los granulocitos neutrofilos con nucleo hinchado (Fig 24 y 25) con nucleo autolizado y protoplasma a punto de romperse (Fig 26), el nucleo autolizado libre que va hacia la formacion de una masa nuclear libre pero que todavia no tiene forma redonda (Fig 27) y finalmente el granulocito neutrofilo intacto tratando de fagocitar la masa libre (Figs 28 y 29) Después de esta observacion no nos queda ninguna duda que la masa amorfa fagocitada es de origen nuclear a partir de un granulocito neutrófilo La unica objeción que podria hacerse a este esquema al parecer tan claro es que el fenomeno del nucleo hinchado y del nucleo autolizado no son específicos del L E ya que los hemos visto aparecer en artritis reumatoideas y otros cuadros afines pero en ninguno de ellos se llega a la etapa de célula del L E

Como hechos complementarios podemos agregar que en 25 casos la velocidad de sedimentación estaba elevada encontrandose cifras superiores a 30 mm en una hora en 21 casos Anemia discreta normocitica y normocromica es decir con los caracteres de las anemias secundarias se encontro en 16 casos Leucopenia por debajo de 5 000 leucocitos se encontro en 9 casos numero normal de leucocitos (5–8 000) se encontró en 10 casos y leucocitosis en 7 casos

Comentario

El estudio de 26 casos de Lupus Eritematoso Diseminado y el de un grupo de enfermedades del mesénquima nos lleva a la conclusion que las células del L E y las rosetas son especificas del Lupus Eritematoso Diseminado y que su hallazgo basta para confirmar el diagnostico de la enfermedad Esta confirmacion debe basarse en la observacion de mas de algun elemento típico ya que las celulas atipicas y la aglutinacion de los leucocitos se presentan en una serie de enfermedades que no corresponden al diagnostico de Lupus Eritematoso Diseminado De gran ayuda para la interpretacion de estos fenómenos es el conocimiento de los síntomas clínicos del enfermo la presencia de una velocidad de sedimentacion elevada unida a una anemia secundaria o a una leucopenia

La naturaleza de estos fenomenos es immunologica y debe estar en relacion con la presencia de anticuerpos anormales similares a los que se encuentran en las anemias hemoliticas y en las trombopenias esenciales Es de interés destacar el incremento de estos fenomenos con las infecciones intercurrentes y su disminucion y aun desaparicion en los casos tratados con Cortisona lo que refuerza aun mas la idea que estos fenomenos son immunologicos A esto puede agregarse el hecho que el factor plasmatico esta contenido en la fraccion gamma globulina del plasma fraccion que esta relacionada intimamente con los anticuerpos

La serie de los numerosos casos hematológicos estudiados en sus proteínas por el método electroforético no puede permitir conclusiones, pero en cambio este comienzo puede ser un estímulo para hematólogos y bioquímicos en el afán que ellos tienen de disipar tantos puntos obscuros

Metodo y Material

La mayor parte de los estudios fueron efectuados en sueros pero en algunos casos especiales se complementó la investigacion con el estudio del plasma del mismo caso

Sistemáticamente se diluyo en la proporcion de 1 × 4 usando siempre el mismo buffer a pH 8 4 y con fuerza ionica de 0 10 M de cloruro de sodio y 0 02 de fosfatos y dializando contra un litro de solucion amortiguadora generalmente durante 24 horas en papel celofán

El instrumental que se ocupa es el aparato de Tiselius modificado por Longsworth con 50 miliamperes durante 3 horas y 3 2 watts

Se usa la célula standard de 11 ml de capacidad y a una temperatura de 1 C

Los patterns obtenidos se proyectan siempre a una misma distancia y equivalente a la raíz cuadrada de 10 en consecuencia el centímetro de longitud se traduce en una amplificacion igual a 3 16 (3 16 × 3 16 = 10)

La ampliación fué medida por el método planimétrico compensador y sus áreas calculadas en unidades abstractas que dan el % relativo de la concentración total de proteínas y a su vez el % relativo de las fracciones De las unidades abstractas o de los % relativos de concentración se obtiene el índice A/G

De las unidades abstractas es posible expresar la concentración absoluta en gramos % y en forma muy aproximada toda vez que se dispuso de los factores empiricos siguientes el promedio de las unidades abstractas del suero equivalente a 56 2 y el del plasma igual a 55 y el segundo factor necesario para esta conversión la proteinemia química normal le sujetos supuestos sanos equivalente en nuestro medio a 7 gramos % de concentracion absoluta

La conversion de las unidades abstractas a gramos % conociendo los dos factores empíricos enunciados se obtiene multiplicando el promedio de las unidades abstractas enunciadas por la constante igual a 1245 para el suero y 1271 para el plasma

De este modo se hace posible calcular la proteinemia en forma aproximativa sin necesidad de recurrir al método de Khjeldahl (Técnica original del Dr Manuel Madrid aun no publicada)

El estudio electroforético de las proteínas en algunas enfermedades hematológicas comprende

1 Estudio estadístico de material humano normal y sus cifras calculadas con desviaciones standard
2 Anemias Por hemorragia aguda por carencia de hierro anemia perniciosa
3 Ictericia hemolítica congénita familiar
4 Policitemia vera
5 Leucemias Leucemia aguda linfoblástica leucemia aguda promonocitica leucemia aguda mielomonocítica leucemia mieloide cronica
6 Retículo endoteliosis leucémica
7 Mieloma multiple
8 Rubéola
9 Mononucleosis infecciosa
10 Enfermedad de Hodgkin linfosarcoma
11 Purpuras Trombopénica atrombopénica

Anemia por Hemorragia Aguda

Solo se presenta un caso (grafico 1265 fig 1) en el que se observa una serina y beta globulina normales en su concentracion y movilidades alfa globulina

VIII 5

Estudio Electroforético de las Proteínas en Algunas Enfermedades Hematológicas

M. MADRID, G. DUCACH y EMILIA BITRAN*

EL INDISCUTIBLE aporte de mayores conocimientos que ha proporcionado el estudio de las proteínas en general y particularmente las del plasma y del suero humano por el nuevo método denominado electroforesis, sin considerar otros procedimientos conocidos como por ejemplo el ultracentrifugado y las técnicas inmunobiológicas de las precipitinas, nos han movido con cierta curiosidad desde hace poco tiempo a pasar en revista los trabajos realizados separadamente por una parte en el laboratorio de electroforesis y por otra en el laboratorio de hematología, ambos departamentos anexos a la Cátedra de Medicina del Prof. Dr. Rodolfo Armas Cruz del Hospital del Salvador de Santiago de Chile.

Con el material acumulado durante poco más de dos años no era posible valorar la experiencia sin un mayor esfuerzo hecho en los últimos cuatro meses buscando material hematológico que al mismo tiempo fuese estudiado por el método electroforético.

Es por esas razones que se señala una serie de enfermedades donde la hematología fuera siempre elemento sustancial del diagnóstico clínico.

Y *así como todavía permanece muy obscura la etiología* de muchas enfermedades hematológicas a pesar del progreso proporcionado por las punciones exploradoras de los órganos hematopoyéticos, así también el origen de las proteínas y sus fracciones no está completamente dilucidado.

Tanto de una de estas ramas como en la otra de la investigación que colabora con la clínica médica deben existir problemas y causales comunes y donde aun la bioquímica con su progreso, su instrumental y dispositivos no ha sido capaz de penetrar.

Hasta ahora la hematología ha vivido en una etapa morfológica por la cual los diagnósticos se hacen a través de una determinada alteración del hemograma o del mielograma. En presencia de una etapa morfológica y entrando en terreno de la físico química, los estudios electroforéticos nos muestran que algunas enfermedades hematológicas, al igual que una alteración típica, presentan una típica modificación del esquema de las proteínas sanguíneas.

La bioquímica está dando valores exactos de orden matemático que permiten encauzar el criterio hacia conclusiones del mismo orden y bien puede ser que esta asociación de estudios mediante la base matemática que la bioquímica proporciona se pueda llegar con el tiempo y por una nueva ruta a valorar lo que hasta ahora es desconocido.

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con excepcion de la fraccion gamma globulina que muestra una movilidad discretamente disminuída

El otro caso (1275) muestra serina y alfa disminuídas con movilidad normal beta normal y gamma discretamente aumentada con movilidad normal En estos dos casos no existe ningun hecho que permita plantear conclusiones

Anemia Perniciosa

Se han estudiado 5 casos de anemia perniciosa (2 hombres y 3 mujeres) dos con anemia en el momento del estudio de sus proteinas y tres sin anemia

El primero es un caso raro y dificil de interpretar (graficos 1160 1172 1174) Se trata de un hombre de 36 años con antecedentes etilicos francos con estado carencial, que ingresa al hospital por una neumonia lobar derecha con 40° de temperatura El primer recuento dio 2 000 000 de globulos rojos con 52% de hemoglobina y un valor globular 1 30 Siete dias mas tarde y habiendo sido tratado con 100 gammas diarias de Vit B_{12} penicilina y transfusiones de sangre modifica su hemograma a 3 400 000 glóbulos rojos y un tercer hemograma cinco dias mas tarde muestra 4 300 000 con 90% de hemoglobina (fig 3)

El grafico electroforetico del suero que coincide con el primer hemograma revela hiposerinemia de 39% con indice A/G de 0 61 y gamma globulina desdoblada en dos fracciones una con 14 5 y la otra con 22 6% relativos beta aumentada a 18% las movilidades moleculares de serina y alfa discretamente disminuídas especialmente de la serina

Dos semanas tarde un nuevo grafico electroforetico del plasma revela casi identica conclusion que el primero El grafico del suero de la misma fecha siempre mostro hiposerinemia índice A/G de 0 54 y con gamma globulina en un solo peak pero con 42 6% de concentracion relativa y con movilidades moleculares normales de gamma y beta pero serina y alfa disminuidas

Un caso aislado (grafico 1290) de anemia perniciosa con anemia de 2 200 000 glóbulos rojos presenta hiposerinemia de 41 2% alfa aumentada beta normal y gamma aumentada y dividida en dos fracciones gamma prima y gamma movilidad molecular en que gamma prima esta aumentada (fig 4)

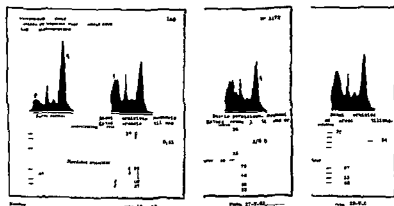


Fig 3 —Anemia perniciosa

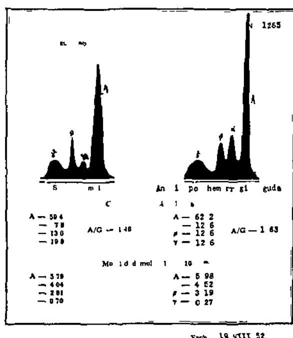


Fig 1 —Anemia por hemorragia aguda

aumentada con movilidad normal y una gamma globulina disminuida en su concentración y en su movilidad molecular Este solo caso no permite conclusiones

Anemia por Carencia de Hierro

Se presentan dos casos (Graficos 1285 y 1275 fig 2) En uno de los casos (1285) las concentraciones en % y la movilidad de las fracciones son normales

Fig 2 —Anemia por carencia de hierro

PROMEDIO DE LA CONCENTRACION [illegible] DE LA PROTEINEMIA EN 57 [illegible] Y 33 PLASMAS HUMANOS [illegible] CON LAS DESVIACIONES [illegible]

ESTUDIO ELECTROFORETICO

57 [illegible] — 33 PLASMAS

	A		B	[illegible]	/[illegible]	A	a	[illegible]	Ø	G	A/G
[illegible] D H Moore	62	10	13	15	1 63	62	8	13	5	12	1 63
Promedio matemático	59 3	7 76	13 0	19 6	1 48	57 4	7 9	12 5	5 8	16 4	1 36
Promedio práctico	59 4	7 8	13	19 7	1 48	57 4	8	12 5	5 8	16 4	1 36
DS O ±	4 58	2 12	3 61	3 32	0 [illegible]	5 51	2 40	2 11	1 91	3 61	0 21
DS M ±	0 60	0 28	0 47	0 44	0 03	0 96	0 41	0 36	0 33	0 62	0 03

DS O = $\sigma = \sqrt{\frac{[illegible]}{[illegible]}}$ $\sqrt{\frac{[illegible]}{[illegible]-1}}$

DS M = $\frac{\sigma}{\sqrt{N}}$

CUADRO 2

Ictericia Hemolítica Congénita Familiar

Se estudian cuatro casos de ictericia hemolítica congénita familiar (I H C F) todos ellos incluídos en el sub grupo de las anemias microc ferocíticas En tres casos la edad fluctua entre 20 y 30 anos y el cuarto caso es una persona de 52 anos

El estudio electroforético indica una concentracion relativa en % de las pro teínas del suero de estos enfermos con un discreto aumento de la gamma globu lina con excepcion del caso 1127 (normal) La sero albumina muy cerca de la cifra normal la relacion A/G también normal

El unico factor constante en forma sugestiva es la concentracion relativa de la alfa globulina disminuída a cifras fuera del limite maximo inferior (Normal 7 8% Mínimo 5 7%)

FIG 5 — Anemia perniciosa sin anemia

PROMEDIO DE LA MOVILIDAD MOLECULAR EN 57
SUEROS Y 33 PLASMAS HUMANOS NORMALES
CON SUS DESVIACIONES STANDARDS

E S T U D I O E L E C T R O F O R E T I C O

	57 SUEROS				33 PLASMAS				
	A	a	B	G	A	a	B	ø	G
Promedio Matemático	5 79	4 04	2 81	0 70	5 51	3 91	2 68	1 87	0 60
Promedio Práctico	5.8	4	2 8	0 70	5 5	3 9	2 6	1 9	0 6
DS O ±	0 67	0 60	0 51	0 36	0 813	0 447	0 37	0 318	0 319
DS M ±	0.09	0.09	0.69	0.04	0.141	0 078	0 06	0 055	0 064

$$DS\ O = \sigma = \sqrt{\frac{\Sigma\ f^2}{N} - k^2} \qquad x = \sqrt{\frac{f}{h-1}}$$

$$DS\ M = \frac{\sigma}{\sqrt{N-1}}$$

CUADRO 1

Comentario En los dos casos de anemia perniciosa con anemia aparece el desdoblamiento de gamma globulina en forma muy nítida especialmente en el caso complicado de neumonia, carencia y etilismo, hecho que no se observa en los casos que se insertan a continuacion

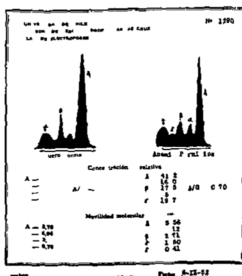


FIG 4 —Anemia perniciosa con anemia

Tres casos de anemia perniciosa en un período en que no se comprobo anemia (gráficos 1228 1238 y 1271) por tratamiento de mantencion con Vit B_1 mostraron lo siguiente todos ellos aumento discreto de gamma globulina con movilidades moleculares que pueden denominarse normales El caso 1238 tiene una seronemia relativamente baja (48%) y gamma 30 9% con indice A/G de 0 92 y movilidad molecular de 0 19

PROMEDIO DE LA CONCENTRACION RELATIVA DE LA PROTEINEMIA EN 57 SUEROS Y 33 PLASMAS HUMANOS NORMALES CON LAS DESVIACIONES STANDARD

ESTUDIO ELECTROFORETICO

57 SUEROS — 33 PLASMAS

	A	a	B	G	A/	A	a		β	G	A/G
D H Moore	62	10	13	15	1 63	62	8	13	5	12	1 63
Promedio matemático	59 3	7 76	13 0	19 6	1 48	57 4	7 9	12 5	5 8	16 4	1 36
Promedio práctico	59 4	7 8	13	19 7	1 49	57 4	8	12 5	5 8	16 4	1 36
DS O ±	4 58	2 12	3 61	3 32	0 6	5 51	2 40	2 11	1 91	3 61	0 21
DS M ±	0 60	0 28	0 47	0 44	0 05	0 96	0 41	0 [illegible]	0 33	0 62	0 03

DS O = σ = $\sqrt{\frac{[illegible]}{[illegible]}}$ $\sqrt{\frac{[illegible]}{[illegible] - 1}}$

DS M = $\frac{\sigma}{\sqrt{N}}$

CUADRO 2

Ictericia Hemolítica Congenita Familiar

Se estudian cuatro casos de ictericia hemolítica congenita familiar (I H C F) todos ellos incluídos en el sub grupo de las anemias microesferocíticas En tres casos la edad fluctua entre 20 y 30 anos y el cuarto caso es una persona de 52 anos

El estudio electroforético indica una concentracion relativa en % de las pro teinas del suero de estos enfermos con un discreto aumento de la gamma globu lina, con excepcion del caso 1127 (normal) La sero albumina muy cerca de la cifra normal la relacion A/G tambien normal

El unico factor constante en forma sugestiva es la concentración relativa de la alfa globulina disminuida a cifras fuera del limite máximo inferior (Normal 7 8% Minimo 5 7%)

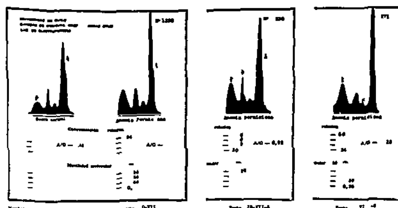


FIG 5 —Anemia perniciosa sin anemia

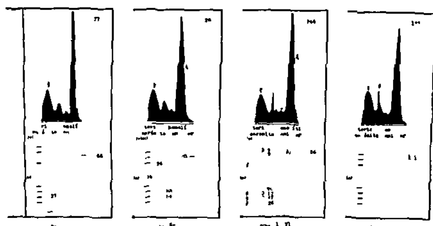


Fig 6 —Ictericia hemolítica congénita familiar

Lo sugestivo de este hecho es la coincidencia entre la hipoalfaglobulinemia y la microesferocitosis Puede decirse que uno de estos fenómenos es el reflejo del otro (fig 6 gráficos 1127 1128, 1244 y 1278)

Policitemia Vera

Se estudian tres casos (gráficos 1226 1264 y 1276) el primero que podría ser una TBC esplénica y que se muestra asociada a una lues con aneurisma aórtico los otros dos con distinta historia clínica un caso en fase terminal y el otro en pleno período de estado

El primer caso muestra profunda alteración de las concentraciones de las proteínas estudiadas electroforéticamente Puede verse la baja de la serina a 30 2 % relativo con índice A C de 0 44 que se traduce en una hiperglobulinemia con 16 4 de alfa y 35 5 de gamma y beta discretamente aumentada Las movilidades moleculares de este caso son serina y alfa bajas beta y gamma normales

De los otros dos casos el de la mieloesclerosis terminal tiene un gráfico electro

LIMITES DE VARIACION DE LA CONCENTRACION RELATIVA EN % Y DE LA MOVILIDAD MOLECULAR DE LAS PROTEINAS DEL SUERO Y PLASMA NORMALES

ELECTROFORESIS

	% SUERO		% PLASMA
A	54 8 — 64	A	51 9 — 62 9
a	5 7 — 9 9	a	5 6 — 10 4
B	9 4 — 16 6	B	10 4 — 14 6
G	16 5 — 3 1	Ø	3 9 — 7 7
		G	12 7 — 19 9

MOVILIDAD μ = 10^{-5} ms

A	5 09 — 6 49	A	4 7 — 6 3
a	3 44 — 4 64	a	3 4 — 4 4
B	2 31 — 3 31	B	2 2 — 3 0
G	0 30 — 1 10	Ø	1 6 — 2 2
		G	0 5 — 0 8

Cuadro 3

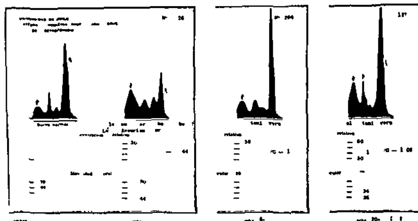


Fig 7 —Policitemia vera

forético que puede catalogarse practicamente como normal tanto en sus concentraciones relativas de proteinas como en sus movilidades moleculares Este caso no tiene hiperglobulinemia en el momento del examen En cambio el caso estudiado en franco periodo de estado de la enfermedad revela unicamente una discreta disminucion de serina y alfa y un aumento de gamma con su movilidad disminuída (fig 7)

Leucemias

El capitulo de las leucemias lo hemos dividido en leucemias agudas con 3 casos linfoblásticos un caso monocitico y un caso mielomonocítico y 2° grupo leucemias mieloides cronicas con 5 casos

De las leucemias agudas linfoblasticas los dos casos (graficos 1221 y 1288, fig 11) tienen hiposerinemia alfa normal Los demas factores de concentración movilidad de las fracciones se comportan caprichosamente

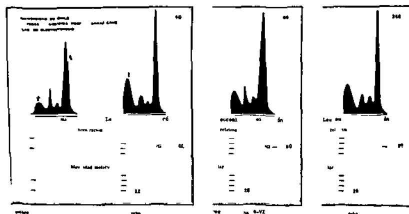


Fig 8 —Leucemia mieloide crónica

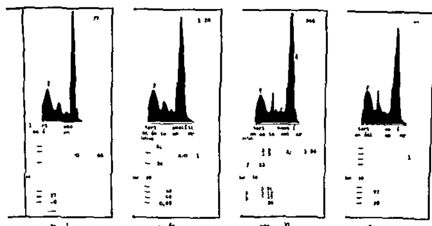


Fig 6 —Ictericia hemolítica congénita familiar

Lo sugestivo de este hecho es la coincidencia entre la hipoalfaglobulinemia y la microesferocitosis Puede decirse que uno de estos fenómenos es el reflejo del otro (fig 6 gráficos 1127 1128 1244 y 1278)

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El primer caso muestra profunda alteración de las concentraciones de las proteínas estudiadas electroforéticamente Puede verse la baja de la serina a 30 2% relativo con índice A G de 0 44 que se traduce en una hiperglobulinemia con 16 4 de alfa y 35 5 de gamma y beta discretamente aumentada Las movilidades moleculares de este caso son serina y alfa bajas beta y gamma normales

De los otros dos casos el de la mieloesclerosis terminal tiene un gráfico electro-

LIMITES DE VARIACION DE LA CONCENTRACION RELATIVA [illegible] Y [illegible] LA MOVILIDAD MOLECULAR DE LAS PROTEINAS DEL SUERO Y PLASMA NORMALES

ELECTROFORESIS

	% SUERO		% PLASMA
A	54 8 — 64	A	51 9 — 62 9
a	5 7 — 9 9		5 6 — 10 4
B	9 4 — 16 6	B	10 4 — 14 6
ω	16 5 — 3 1	ø	3 9 — 7 7
		G	12 7 — 19 9

MOVILIDAD μ = 10 $^{-5}$ cms

A	5 09 — 6 49	A	4 7 — 6 3
a	3 44 — 4 64	a	3 4 — 4 4
B	2 31 — 3 31	B	2 2 — 3 0
G	0 30 — 1 10	ø	1 6 — 2 2
		G	0 5 — 0 9

Cuadro 3

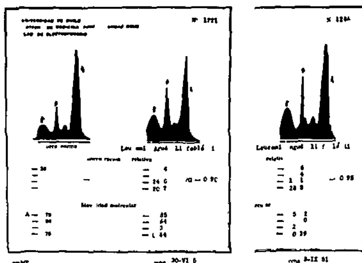


FIG 11 —Leucemia aguda linfoblástica

normal de proteínas pero las velocidades moleculares aumentan a cifras normales

Este enfermo a raíz de la radioterapia desarrolló una anemia aguda con test de Coombs positivo que ha sido controlada con transfusiones y cortisona

Leucemia Mieloide Cronica

Se estudiaron 5 casos

El grafico # 1140 (fig 8) que corresponde al grupo 1140 1184 y 1266 muestra una serina disminuída y una gamma globulina aumentada con movilidad molecular disminuida en todas las fracciones Los otros dos graficos de la misma figura 8 son del mismo enfermo el primero (1184) despues de un tratamiento con TEM (7 800 leucocitos) muestra % normales de todas las fracciones con una movilidad molecular disminuida El segundo grafico (1266) cuando el enfermo

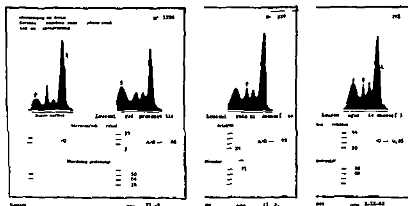


FIG 12 —Leucemia aguda promonocítica y leucemia aguda mielomonocítica

Fig 9 —Leucemia mieloide crónica

De las leucemias agudas del grupo monocítico (gráficos 1224 1257 y 1291 fig 12) el primero (1224) mostró una hiposerinemia de 39 8% con índice A/G de 0 66 y globulinas aumentadas especialmente alfa y gamma con beta normal La movilidad molecular de serina y gamma disminuída y las fracciones alfa y beta normales Otro caso de la variedad mielomonocítica (1257 y 1291) con hiposerinemia alfa y gamma aumentadas beta normal dieron movilidades moleculares normales

Otro caso aislado estudiado después de haber sido radiado con radioterapia profunda y en pleno tratamiento con cortisona (200 mgs por día) fue estudiado electroforéticamente en dos oportunidades (gráficos 1240 y 1282 fig 13) En el primer gráfico se encuentra concentración relativa de proteínas normales velocidades moleculares disminuidas En el segundo gráfico persiste la concentración

Fig 10 —Retículo endoteliosis leucémica

de Hodgkin tienen profunda alteración del estado general y principalmente anemia

Retículo Endoteliosis Leucémica

Se presenta separadamente un caso de retículo endoteliosis leucemica crónica con dos trazados electroforeticos Ambos graficos muestran profunda alteracion de la imagen fotográfica y su expresión en % relativo de sus fracciones (graficos 1170 y 1237 fig 10)

En el primero de los graficos aparecen las movilidades moleculares disminuidas en forma franca en el segundo la movilidad tiende a normalizarse Tanto en uno como en otro de los patterns electroforéticos la gamma se presenta aumentada a 51 5 y 57 6 % relativo, respectivamente cifras solo comparables con lo observado en el mieloma multiple Ambos graficos muestran movilidades moleculares de 0 26 y 0 24 que quedan fuera del limite inferior tolerable de la movilidad de gamma globulina normal

Mieloma Multiple

De mieloma multiple se estudiaron 13 enfermos (11 hombres y 2 mujeres) cuyas edades fluctuaban entre 36 y 80 anos El diagnostico en todos ellos se confirmo por la existencia de un numero aumentado de plasma Zellen en la medula osea los cuales habitualmente tenian caracteres de inmadurez Los estudios electroforéticos demostraron alteraciones de dos órdenes aumento de la concentracion relativa en % de algunas de las fracciones y una variacion de la movilidad molecular

Con excepcion de un caso todos acusaron hiperproteinemia

El unico caso de hipoproteinemia era a la vez una asociacion con amiloidosis

Uno de los 13 casos (grafico 1166) con hiperserinemia (índice A/G de 2 70) presenta movilidad molecular de la serina disminuida

Un segundo caso (grafico 1116) donde la fracción aumentada es la alfa globulina con hipoproteinemia y movilidades moleculares normales En este caso se comprobo la asociacion del mieloma con una amiloidosis generalizada confirmada con la autopsia

Un tercer caso (gráfico 240) con beta globulina muy aumentada (58 1 %) y movilidades moleculares del orden normal (fig 14)

Tres casos (graficos 263 813 y 824) con las siguientes caracteristicas gamma aumentada en forma considerable con movilidad molecular nula (fig 15)

Cuatro casos (graficos 1073 1117 1136 y 1173) en que la fraccion aumentada se denomina M componente y se la ubica en cuanto a su movilidad molecular entre la gamma y la beta globulina (fig 16) El caso 1117 fué repetido dos meses mas tarde (grafico 1139 fig 18) habiéndose tratado durante ese periodo con uretano El resultado electroforético muestra en primer lugar siempre un aumento del componente M (63 6 %) que pasa a ubicarse a la altura de la gamma normal

Un grupo de tres casos (gráficos 1060 1168 1177 A fig 17) con la fraccion

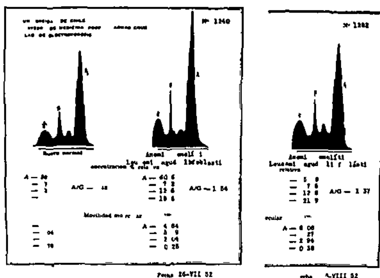


FIG 13 —Leucemia aguda linfoblástica anemia hemolítica

manifestaba una nueva alza de los globulos blancos de 51 000 muestra serina disminuida beta normal y alfa y gamma aumentadas Las movilidades moleculares se encuentran normales con excepcion de que gamma esta disminuída en su velocidad de migracion

Los trazados 1229 y 1241 (fig 9) son de un mismo enfermo el primero antes y el segundo después de un tratamiento con rayos x En el 1229 (153 000) se observa una alfa aumentada y una gamma disminuída y en cuanto a la movilidad molecular es normal con excepcion de gamma que tiene movilidad molecular discretamente disminuída En el segundo grafico (1241) (3 300 leucocitos) lo unico anormal es una gamma discretamente disminuida *su movilidad con el tratamiento se ha hecho normal*

El grafico 1256 (fig 9) es tomado a un enfermo después de 3 meses de tratamiento con rayos x y el hemograma mostraba 13 400 leucocitos En el gráfico se observa una serina aumentada y una beta disminuída en su % el resto es normal lo mismo que la movilidad de todas las fracciones

El ultimo caso (1292 fig 9) muestra de patologico una beta aumentada en su % la sangre es tomada sin tratamiento cuando la enferma tenía 250 000 leucocitos

Comentario En las leucemias mieloides cronicas no es posible llegar a ninguna conclusion definida porque las variaciones son poco significativas y no son constantes

Llama la atencion el caso 1229 y 1184 en que la movilidad molecular de gamma se hace normal después del tratamiento con rayos x en cambio el grafico 1184 (fig 8) que es con cantidad normal de globulos blancos en un enfermo después de un tratamiento con TEM presenta movilidad disminuída de sus fracciones En resumen no es posible anotar conclusiones definidas

Tambien hay que considerar que estos enfermos al igual que en la enfermedad

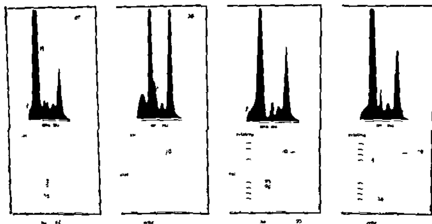


FIG. 16.—Mieloma múltiple

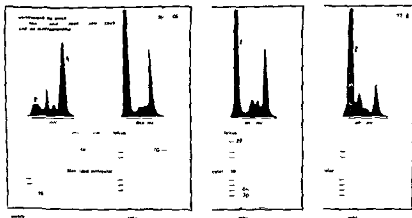
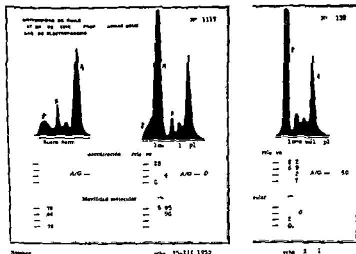


FIG. 17.—Mieloma múltiple



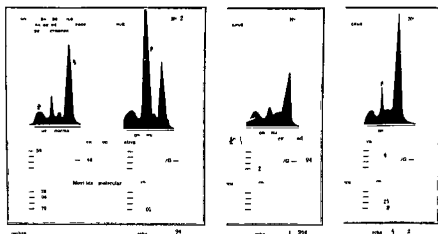


Fig 14 —Mieloma multiple

gamma enormemente aumentada y con movilidad molecular dentro de los límites de la gamma normal

Comentario Rundles y colaboradores [10] estudiando con buffer de barbital a pH 8 6 y con constantes de sedimentacion por ultra centrifugado concluye estimando que las células plasmaticas del mieloma multiple estan asociadas a una anormalidad en la sintesis de las proteinas

Los componentes anomalos aparecen en el mieloma multiple en cantidades suficientes como para producir hiperproteinemia y la mayoria excretan proteinas de Bence Jones en la orina

La relacion entre la proliferacion de plasma Zellen y la produccion de proteinas anomalas y su relacion con los componentes del suero con las proteínas de Bence Jones es todavía obscura Es asi como en el suero anomalo las proteínas urinarias varian en cada enfermo tanto en movilidad electroforetica solubilidad en soluciones salinas en peso molecular y reaccion frente a las precipitinas

Sugieren los autores que los aumentos de la gamma globulina en el suero o

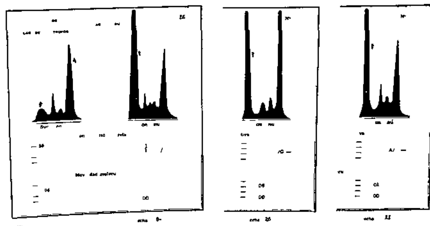


Fig 15 —Mieloma multiple

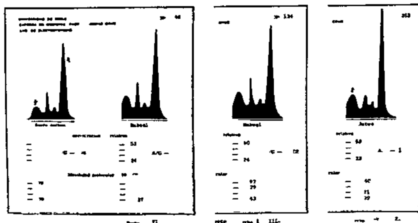


FIG 19 —Rubeola

Rubeola

Se presenta a continuacion de los mielomas una serie de tres casos de rubeola y en atencion a que en esta enfermedad infecciosa aguda aparecen plasma Zellen aumentados en la sangre periferica uno con 9% (grafico 1246) otro con 18% (grafico 1247) y el tercero sin plasma Zellen en el torrente circulatorio

El estudio electroforetico de las proteinas y sus fracciones en general no revela otra alteracion sino una discreta disminucion de serina y una gamma globulina aumentada que puede ser significativa a pesar de que en todas las enfermedades infecciosas se observa un aumento de esa fraccion

La movilidad molecular corresponde en los tres casos a los limites normales (fig 19)

Mononucleosis Infecciosa

Dos casos de suero de mononucleosis infecciosa (graficos 1223 y 1242) y un caso de plasma (grafico 1234) son estudiados con las mismas tecnicas ya expuestas y considerando los trabajos de Stirling[14] sobre el mismo tema

Un caso de suero (1223) con reaccion Paul Bunnell negativa y los otros dos con esta reaccion positiva

La unica observacion que merece este grupo hematologico se refiere al caso de plasma con un aumento franco de la alfa globulina que se eleva a un 13%

Las demas fracciones tanto en su concentracion como en su movilidad molecular se las encuentra con muy insignificantes variaciones dentro de los limites normales (fig 20)

Enfermedad de Hodgkin

Se estudian 5 casos (1191 y 1272 1220 fig 21 y graficos 1279 1281 y 1287 fig 22) 4 hombres y 1 mujer En todos los patterns electroforéticos se encuentra el unico factor comun en la hiposerinemia encontrandose las globulinas normales o aumentadas La movilidad molecular estaba dentro de los limites normales

plasma del mieloma multiple puede representar anticuerpos a las proteinas de Bence Jones Serían ellas posiblemente derivadas de esos constituyentes anomalos

Finalmente, los autores creen que los plasma Zellen podrían producir mas de un tipo de proteina anomala

Los autores de este trabajo después de pasar revista a la literatura sobre mieloma multiple y considerando los hechos ya demostrados de las enormes variaciones en la concentracion relativa de las proteínas anomalas la diferente movilidad molecular que ellas acusan especialmente la gamma globulina o M componente que se comporta con una movilidad de igual a 0, más baja que lo normal o igual a la del fibrinogeno etc , los trabajos de Moore y colaboradores, que determinan porcentajes insignificantes de proteinas de Bence Jones formando parte del componente anomalo cuya cantidad no es superior a 0 2 %, estiman que hay sin duda alguna un trastorno funcional en la produccion de este tipo de proteína por alteracion de uno de los centros generadores de ellas como se de muestra por los trastornos oseos y medulares a través de la radiografia y de la hematologia Hay que recordar como complemento de esta hipotesis que las proteínas de Bence Jones tienen peso molecular inferior a la sero albumina (30 000) y, sin embargo acompanan o migran junto con proteínas que tienen pesos moleculares de 160 000 como lo es el peso molecular de la gamma globulina

El mecanismo íntimo estaria ligado al mosaico electronico que representa la cadena de aminoacidos y polipéptidos que forman las moléculas protéicas cuyas cargas positivas o negativas le dan su característica y podriase agregar su especificidad

De acuerdo con esta hipotesis serian las causas anteriormente descritas las que provocarían diferentes puntos isoeléctricos de las proteínas anomalas cuya manifestacion se traduce en una diversa movilidad molecular en un campo electroforetico

Se ha observado por ejemplo en un caso de mieloma multiple que el trata miento con uretano modifica la movilidad molecular en un componente que migra con mayor velocidad que la gamma globulina y que más tarde después de recibir una dosis de esa droga ese componente anomalo se ubica a la altura de la gamma globulina normal

En otro caso de una leucemia mieloide cronica el tratamiento con rayos x provoca una modificacion de la movilidad molecular de la gamma globulina volviéndola a la normalidad y en cambio otro enfermo también de leucemia mieloide cronica tratado con TEM disminuye la movilidad molecular de sus fracciones de proteinas

Muchas otras podrian ser las consideraciones que debieran incluirse en este trabajo pero como la mision de los autores se ha concretado a relatar los hechos encontrados en este estudio comparativo de lo que acontece en las alteraciones morfologicas de los estudios hematologicos con lo que sucede en cuanto al comportamiento de las proteinas de los mismos enfermos no se hace posible mayores discriminaciones en esta materia tan compleja

Las variaciones que se anotan en las concentraciones relativas son de orden mínimo

En un caso con 2 trazados electroforéticos, uno antes de iniciar tratamiento con TEM y el otro dos meses después de terminado este tratamiento solo se aprecia baja de serina y regular aumento de gamma con movilidad molecular normal de todas las fracciones

Dentro de este grupo se incluye un caso de linfosarcoma de una joven de 17 anos cuyo diagnostico se comprobo con la autopsia Solo llama la atencion la baja de serina alfa y gamma aumentada beta dentro de limites normales

Vale la pena senalar la presencia de una anemia de 2 700 000 por ml³ Las movilidades moleculares estaban disminuidas

Hay que tener presente por lo tanto que estos enfermos ademas de su altera cion ganglionar tienen anemia y profundo trastorno de su estado general

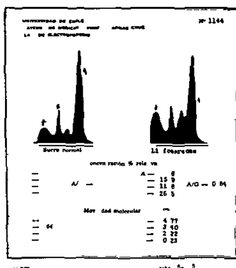


Fig 23 —Linfosarcoma

Purpuras

Pupura Trombopenico Se estudiaron cuatro casos de purpura trombopénico idiopatico dos de ellos con esplenectomia y uno de estos dos estudiado inmediata mente después de la intervencion quirurgica y dos meses mas tarde El primer grafico de este ultimo caso muestra serina discretamente disminuida y con movilidad molecular normal En el segundo grafico persiste la baja de la concen tracion de la serina y aumento discreto de las fracciones alfa y beta gamma se encuentra normal La movilidad molecular dio expresiones normales Es nece sario hacer constar que esta enferma en su periodo pre operatorio recibio nu merosas tran fusiones de sangre El tercer grafico practicado 4 meses despues de la esplenectomía revela que la hiposerinemia persiste con tendencia a bajar que la alfa globulina disminuye a limites tolerables como normales y que la beta

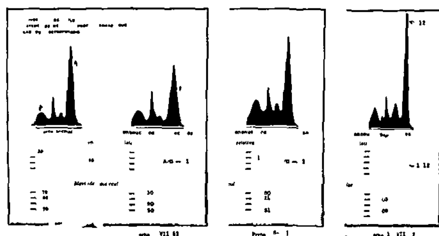


Fig 20 —Mononucleosis infecciosa

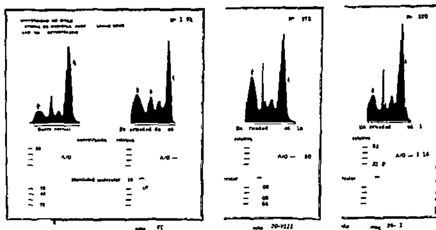


Fig 21 —Enfermedad de Hodgkin

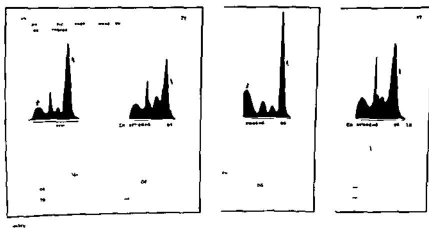


Fig 22 —Enfermedad de Hodgkin

Las variaciones que se anotan en las concentraciones relativas son de orden mínimo

En un caso con 2 trazados electroforéticos uno antes de iniciar tratamiento con TEM y el otro dos meses después de terminado este tratamiento solo se aprecia baja de serina y regular aumento de gamma con movilidad molecular normal de todas las fracciones

Dentro de este grupo se incluye un caso de linfosarcoma de una joven de 17 años cuyo diagnóstico se comprobó con la autopsia Solo llama la atención la baja de serina, alfa y gamma aumentada beta dentro de límites normales

Vale la pena señalar la presencia de una anemia de 2 700 000 por ml³ Las movilidades moleculares estaban disminuidas

Hay que tener presente por lo tanto que estos enfermos además de su alteración ganglionar tienen anemia y profundo trastorno de su estado general

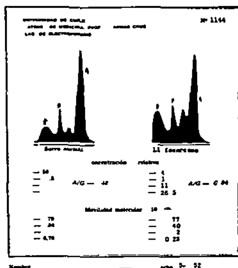


FIG 23 —Linfosarcoma

Purpuras

Pupura Trombopenico Se estudiaron cuatro casos de purpura trombopénico idiopático dos de ellos con esplenectomía y uno de estos dos estudiado inmediatamente después de la intervención quirúrgica y dos meses más tarde El primer gráfico de este último caso muestra serina discretamente disminuída y con movilidad molecular normal En el segundo gráfico persiste la baja de la concentración de la serina y aumento discreto de las fracciones alfa y beta gamma se encuentra normal La movilidad molecular dio expresiones normales Es necesario hacer constar que esta enferma en su período pre operatorio recibió numerosas transfusiones de sangre El tercer gráfico practicado 4 meses después de la esplenectomía revela que la hipo serinemia persiste con tendencia a bajar que la alfa globulina disminuye a límites tolerables como normales y que la beta

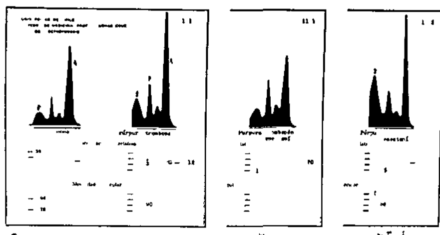


FIG 24 —Purpura trombopénico

y especialmente la gamma globulina aumentan bajando el índice A/G a 0 74 Las movilidades moleculares son normales en los dos primeros gráficos e igualmente en el tercero con excepción de la gamma que junto con aumentar su % disminuye su movilidad de 0 78 a 0 65 y a 0 29 (fig 24, gráficos 1118 1131 y 1262) Se anotan estas cifras porque es extraño observar que al tiempo de producirse aumento de la gamma globulina la movilidad disminuye hecho que se comenta globalmente y en particular en el capítulo correspondiente de este trabajo

El segundo caso (grafico 1233) esplenectomizado un año antes reingresa al hospital por nueva crisis trombopenica y TBC ganglionar No obstante podría considerarse muy similar al anterior en su ultimo gráfico en lo que se refiere a la concentración de proteínas índice A/G y gamma globulina con una cifra con

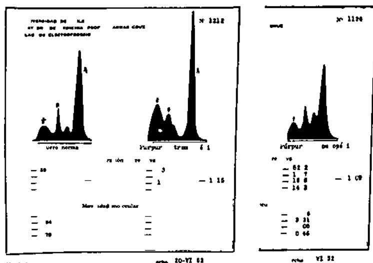


FIG 25 —Purpura a trombopénico

solo 1 % de diferencia de la anterior Las movilidades moleculares coinciden dentro de las cifras normales Los otros dos casos de tipo crónico en enfermos de 55 y 65 años de edad (gráficos 1232 1252 y 1267) tienen entre sí gran similitud y con los casos anteriores Uno de estos tiene dos trazados electroforéticos en el momento de producirse una remisión y tratado con Cortisona, pero sin aporte significativo

Púrpura Atrombopénico En dos casos de púrpura atrombopénico (16 y 19 años de edad) se presentan alteraciones que no pueden ser interpretadas y donde el único hecho anormal común es la serina discretamente disminuida siendo las demás fracciones caprichosas en su concentración

Las movilidades moleculares de un caso (gráfico 1212) son normales con la excepción de la *movilidad de la gamma que se encuentra aumentada* El *otro* (gráfico 1124) presenta movilidades disminuidas en todas las fracciones menos gamma que se encuentra normal (fig 25)

Discusión

Con referencia a lo expresado sobre mieloma múltiple hay que dejar establecido que no se trata de un capítulo exclusivo dada la importancia que esta materia se merece Se ha considerado simplemente uno de los cuadros hematológicos que en el Servicio de Medicina del Prof Dr Rodolfo Armas Cruz ha reunido el mayor número de casos con estudios hematológicos repetidos radiología y necropsia

Para la *interpretación de sus resultados han sido considerados debidamente* los trabajos de W S Adams y colaboradores D H Moore Kabat y Gutmann, Jodocus P Hoessly y L D Greenberg Wurman y Wunderlicht etc y estamos ciertos que las conclusiones tienen gran similitud con los resultados obtenidos por dichos investigadores

Las diferencias que pueden destacarse obedecen a las distintas técnicas empleadas por unos u otros, pero tanto la presencia de fracciones anómalas como las variaciones de las movilidades moleculares de las proteínas estudiadas son también evidentes

La clasificación en grupos depende del criterio individual El primer hecho sustancial que motiva los resultados aparentemente diferentes ha sido la dilución distinta y el diferente pH de las soluciones amortiguadoras que fueron empleadas en estas investigaciones

Adams y colaboradores sacrifican las altas diluciones para obtener mediante un pH más alcalino y un mayor tiempo de migración imágenes o patterns electroforéticos que ilustran en forma muy precisa las diversas fracciones anómalas en cuanto a cantidad y en cuanto a movilidad

Con un pH de 7 4 a base de fosfatos y con tiempo standard de 3 horas se logra obtener patterns que si bien es cierto no son tan minuciosos como los obtenidos por Adams son lo suficientemente útiles para hacer las interpretaciones que se comenta

Es posible que el empleo de los barbituratos como solución amortiguadora a base de un pH 8 6 suministrado por el Veronal en la concentración necesaria

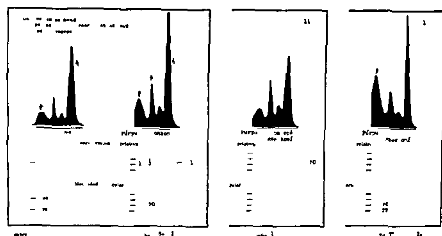


Fig 24 —Purpura trombopénico

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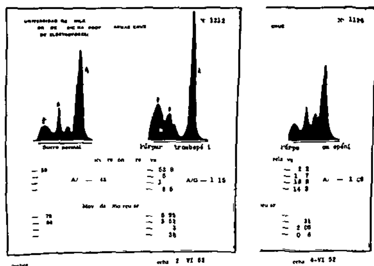


Fig 25 —Purpura trombopénico

Estos casos sugieren que la proteinemia de Bence Jones muy acentuada solo debe producirse en los mielomas en muy pocos casos pero con gran significado clinico

Shapiro y colaboradores[11] en 1943 investigaron la composicion de una proteina viscosa por medio de la dialisis contra agua destilada y obtenida del plasma del mieloma multiple

De esos estudios se llega a la conclusion de que es posible que el componente viscoso sea una globulina del suero que reacciona frente a otro componente del mismo suero

Con referencia a este mismo componente denominado anomalo o M componente Stirling y Reimer[14] en 1949 cita casos similares descritos tambien por Adams y colaboradores que muestran un M componente en la cirrosis hepatica

M Madrid en 1948 presento al Congreso Sudamericano de Quimica un hallazgo de un componente anomalo en la cirrosis hepatica y lo cataloga como un anti cuerpo resultante de un antigeno desconocido Este componente aparecio en cirrosis hepaticas graves con la siguiente frecuencia 9 veces en una serie de 23 casos de suero de cirrosis y 8 veces en una serie de 15 casos de liquidos asciticos desfibrinizados

En las anemias de células falciformes los resultados de los estudios electroforéticos de suero y plasma de Fenichel Watson y Eirich donde la investigacion se hizo en 15 casos se encuentra hiposerinemia en 13 12 con gamma globulina aumentada 3 con beta globulina aumentada y en 12 inversion del indice A/G El fibrinogeno se encontro aumentado en 8 de los 10 casos en que fue determinado

Las anomalias que aparecen en estas investigaciones no son especificas segun los propios autores quienes estiman que la reaccion es debida a trastornos tisulares producidos por el proceso de sickling en varios organos especialmente en el higado

Con referencia al Hodgkin Rottino y colaboradores estudian 33 sueros en 13 encuentran serina normal en 10 casos alfa y beta globulina elevada E te grupo comprende estados terminales anemicos y caquecticos y en 7 casos encuentra gamma globulina elevada

Los 5 casos de Madrid y colaboradores muestran a diferencia de Pottino y colaboradores hiposerinemia y globulinas aumentadas

Un factor no considerado por los investigadores citados es la movilidad molecular de las fracciones de proteina

Conclusiones

Entre otras de las conclusiones de orden general podemos anotar como fundamento para ella ciertos hechos conocidos y demostrados de la importancia que tiene el método que se emplee en e te tipo de investigacion y donde los diferentes centros de trabajo prefieren ciertas tecnicas

El caso mencionado de Hoessly y Greenberg es una muestra evidente de que el empleo de diversas sustancias químicas en las soluciones amortiguadoras es causal de resultados diferentes en un mismo centro de investigacion y en relacion con los trabajos similares de otros laboratorios

pueda provocar un traumatismo molecular capaz de alterar la estructura electroquímica de las proteínas, colocándolas a mucho mayor distancia de su punto isoeléctrico normal

Una demostración de lo que puede suceder en la estructura de las proteínas que se refleja más tarde en los estudios electroforéticos es lo acontecido en un caso de mieloma asociado a amiloidosis citado por Jodocus Hoessly y Greenberg[4]

Estudiando el comportamiento de las proteínas por el método electroforético con soluciones amortiguadoras de barbituratos a pH 8 6 estos autores obtienen una imagen electroforética con una beta globulina Al ser estudiado el mismo caso con una solución amortiguadora con el mismo pH a base de solución buffer de citrato obtienen un trazado electroforético con 2 beta globulina

No está demás citar esas investigaciones y el aspecto con que ellas han sido analizadas Por ejemplo Adams y colaboradores en 61 casos de mieloma múltiple estudiados a través del plasma con hematología, aspecto clínico laboratorio evolución diagnóstico diferencial y tratamiento se hace visible la diferencia de los trabajos realizados por Moore y colaboradores que informan solamente de 7 casos de mieloma múltiple estudiados desde el punto de vista electroforético del ultra centrifugado con constante de sedimentación estudio químico de las proteínas y finalmente con el método inmuno químico de las precipitinas cuantitativas

Adams trabaja con pH 8 6 a base de barbitúricos y Moore y colaboradores a base de fosfatos a pH 7 4

Si bien es cierto que las conclusiones son semejantes en cuanto a las movilidades moleculares la concentración de las proteínas y la presencia de fracciones anómalas es diversa

Los trabajos de esta Cátedra coinciden con Adams y colaboradores que presenta tres casos de mieloma múltiple con un componente anómalo tanto en cuanto a su cantidad como en lo referente a su movilidad molecular Estos tres casos no tienen movilidad molecular disminuída sino igual a 0 Otro grupo donde la movilidad molecular es menor que la mínima movilidad de gamma y si se considera que nuestras cifras se anotan en los gráficos respectivos y en % relativos puede ser denominado M y varía en su movilidad molecular entre la gamma normal y la betaglobulina normal y en consecuencia puede aparecer con la movilidad del fibrinógeno o del ya bien conocido componente M

En la investigación de Moore y colaboradores hay dos casos con acentuada hiperglobulinemia debida al componente gamma estudiada por el método de Howe y ultra centrifugado En los dos casos el tamaño de las moléculas son del orden de la gamma globulina normal

Con el método cuantitativo de las precipitinas se encuentra solamente un 0 2% de proteínas de Bence Jones con concentración tan baja que no puede ser descubierta por otro método

Los autores estiman además que esto acontece en la mayoría de los mielomas

En otro caso de mieloma estudiado también por las precipitinas cuantitativas se encuentra un M o beta globulina con proteínas de Bence Jones en cantidades insignificantes

In these days of immunohematology in which together with morphological variations and differences in the percentages of blood elements harmful circulating antibodies are found as happens in Werlhof s disease and in acquired hemolytic anemias in which corti sone by unknown mechanisms is capable of producing remissions in diseases as different as hemolytic anemia lymphosarcoma acute leukemia disseminated erythematosus lupus etc we have thought it of interest to gather together our material which leads to the observation that frequently in hematological diseases there exist variations of the electro phoretic graphs of blood proteins as much in the *proportions of the different fractions as* in the molecular mobility of its components

The cases considered are not many but they have been studied from all the necessary angles to permit the affirmation of the diagnosis The hematology the radiology the spe cific reactions and even the pathological anatomy contribute in this work

We do not enter into the field of electrophoretic technique

The results of the study of the 100 graphs of normal individuals will also be shown (56 cases of serum and 48 of plasma) these results have been used as a basis to appreciate the variations of our series of hematological cases

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VIII communication 1

Contribution to the Study of the Genesis of White Blood Cells

RAIMUNDO JUAN ROVERE

In the adult mammal the lymphocyte is a persistent embryonic cell and the lymphatic no le i physiological container

The bone marrow is the adult lymphoid organ where the lymphocyte becomes multi nucl polyploid originating the granulocytic series

The lymphoblast and myeloblast are lymphocytes in primary endomitosis and not prim r cells

No obstante, hay un grupo de enfermedades hematológicas que a pesar de su escaso número muestran factores constantes que luego deberán controlarse con una casuística más numerosa. Es el caso de la ictericia hemolítica congénita familiar donde el comportamiento de alfa globulina es sin duda sugestivo. Los 4 casos estudiados muestran una franca disminución de esta fracción.

En orden general es posible anotar el hecho que se observa en la enfermedad de Hodgkin, donde el factor constante, sin excepción, es la hiposerinemia.

Llama la atención en el caso de las anemias perniciosas que las dos únicas anemias perniciosas con anemia presentan doble gamma globulina, en cambio en otros tres casos de anemia perniciosa sin anemia solo revelan discreto aumento de gamma con algunas alteraciones de la movilidad molecular.

El único caso de retículo endoteliosis leucémica muestra una concentración de gamma globulina solo comparable a la concentración que se observa en los mielomas.

En la rubéola, enfermedad estudiada electroforéticamente en cuanto a su concentración de proteínas y sus fracciones solo por el hecho de mostrar en la sangre periférica plasma Zellen, demuestra únicamente una discreta disminución de la serina con discreto aumento de la gamma, hecho común a todas las enfermedades infecciosas.

La misma dificultad se presenta al tratar de interpretar lo que ocurre con las movilidades moleculares en esta miscelánea de enfermedades hematológicas.

Un hecho evidente ya demostrado es el comportamiento de la gamma globulina del mieloma que tiene una movilidad igual a 0 o disminuída a los límites inferiores de lo normal.

Es del caso citar un mieloma no incluido en esta serie de 13 por haberlo estimado defectuoso, pero bien analizado puede estimarse que se trataba de un mieloma a gamma globulina con movilidad molecular inferior a 0, o sea una gamma que migró en sentido contrario.

Nada de extraño puede tener el caso en cuestión si se considera que de los 13 mielomas que se comenta en este trabajo 3 tienen movilidad igual a 0.3 en el límite inferior a lo normal, uno (mieloma o serina) con movilidad molecular disminuída, lo que vale decir, en suma, que en esta enfermedad con componentes anómalos de los sistemas generadores de proteínas existiría un mecanismo físico químico capaz de generar moléculas proteicas con puntos iso eléctricos diferentes a lo normal.

Tal como se dijo al comienzo, la investigación en esta nueva etapa funcional de la hematología requerirá de una prolija observación y técnicas minuciosas para llegar, a través de una casuística numerosa, a interpretar las alteraciones de la compleja estructura de las moléculas de proteínas que se estudian por estos nuevos métodos para poder asignarles algún valor de orden constante que englobe el criterio moderno de la físico química o bio química que debe marchar al mismo compás en el progreso que persigue la hematología.

Electrophoretic Curves in Some Hematological Diseases

About 100 electrophoretic graphs studied in various disturbances of hemopoietic organs are presented.

La Acción de la Asociación Cobalto Hierro en las Anemias Ferroprivas Necatoriásicas Graves

Se describen 20 casos de anemia ferropriva necatoriásicas graves Los pacientes ingresaron al Hospital con síntomas cardiacos muy acentuados globulos rojos por debajo de dos millones y hemoglobina fluctuando de 5 a 1 gramo Fueron tratados con sulfato ferro o y nitrato de cobalto por vía oral obteniéndose curacion completa en un término de 30 días Los pacientes controles con el mismo cuadro anémico y tratados con hierro tardaron 2½ a 5 meses en recuperarse completamente

Se discute la posibilidad de que las sales de cobalto favorezca la utilización del hierro por los órganos hemopoyéticos y su transformación en hemoglobina

VIII communication 3

Cobalt as an Anti anemic Agent in Pre and Postoperative Cases of Reconstructive Surgery

MIGUEL LAYRISSE and DOMINGO LUCCAS*

Ten cases in which it was necessary to carry out reconstructive surgery are described During the pre and postoperative period cobalt salts were administered orally a slight polycythemia being obtained (red cells 5 to 6 millions hemoglobin 15 to 18 Gm %) The grafts caught in nearly all the cases and the healing took place in much less time as compared with the other group of patients with the same type of lesions who received the usual treatment for these cases (liver extract whole blood and plasma transfusions amino acid and protein rich diet) The patients returned to the normal red cell count and hemoglobin in the days following the suspension of the cobalt

Cobalto como Antianémico en el Pre y Post operatorio de los Casos de Cirugía Reconstructiva

Se describen 10 casos en los cuales fué necesario practicar cirujía reconstructiva Durante el pre y post operatorio se administraron sales de cobalto por via oral obteniéndose ligera policitemia (glóbulos rojos 5 a 6 millones hemoglobina de 15 a 18 grms %) Los injertos pegaron en casi su totalidad y la cicatrización se efectuó con mayor rapidez al ser comparados con otro grupo de enfermos del mismo tipo de lesiones en los cuales se administro la terapeutica habitual para estos casos (extracto hepático transfusiones de sangre total y plasma dieta muy rica en proteínas y aminoácidos) Los enfermos volvieron a su cifra habitual de glóbulos rojos y hemoglobina en los días que siguieron a la suspension del cobalto

* Cátedra de Clínica Médica de la Universidad Central Caracas Venezuela

Biology has still not explained the existence of the two types of division in the same tissue

Our work brings forth an hypothesis applicable to comparative zoology and modern cytology We here propose a classification of leukemias according to our hypothesis

1 Embryonic permanent leukemic leukosis due to normoendopolyploid proliferation of the lymphocytes (lymphatic leukemias)

2 Leukosis due to arrest of the multiendopoliploid function of the lymphocytes towards the myeloid series nearly always acute (acute myelogenous leukemia)

3 Leukemic leukosis due to alterations of the multiendopolyploid function of the lymphocytes (chronic myelogenous leukemias)

Contribucion al Estudio de la Genesis de las Celulas Blancas de la Sangre

En el mamifero adulto el linfocito es una célula embrionaria persistente y el ganglio linfático es su acantonamiento fisiológico

La médula osea es el organo linfoide adulto donde el linfocito se hace multiendopoliploide dando las series granulosas

El linfoblasto y mieloblasto son linfocitos en endomitosis primaria y no células primordiales

La biología aun no ha explicado la existencia de los dos tipos de división en un *mismo tejido*

Nuestra comunicación constituye una hipótesis de trabajo con aplicación a la zoología comparada y a la moderna citología

Como corolario proponemos una clasificación de las leucemias de acuerdo a nuestra hipótesis

1 Leucosis leucémicas permanentes embrionarias por multiplicación normoendoploide del linfocito (actuales leucemias linfáticas)

2 Leucosis por detencion de la función multiendopoliploide del linfocito hacia la serie mieloide casi siempre *agudas (actuales* leucemias mieloides criptogeneticas agudas)

3 Leucosis leucémicas *por alteracion de la* funcion multiendopoliploide del linfocito (actuales leucemias mieloides cronicas)

VIII communication 2

Effect of the Association of Iron Cobalt in the Severe Iron Deficient Hookworm Anemias

*MIGUEL LAYRISSE and H CEDRADO**

20 cases of severe iron deficient hookworm anemia are described The patients entered the Hospital with very evident cardiac symptoms red cell count below 2 000 000 and hemoglobin fluctuating between 5 and 1 Gm

They were treated with iron sulfate and cobalt nitrate the cure was effected in a period of thirty days The control patients with the same anemic picture receiving simple treatment with iron took two and a half to five months to cure

The possibility that cobalt salts favor the utilization of iron by the hemopoietic organs and its transformation in hemoglobin is discussed

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La Acción de la Asociación Cobalto Hierro en las Anemias Ferroprivas Necatoriásicas Graves

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